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Environmental Quality Through Transdisciplinary Collaboration

ABSTRACT BOOK

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Society of Environmental Toxicology and Chemistry Europe (SETAC Europe)

ABSTRACT BOOK
SETAC Europe 27th Annual Meeting

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This book compiles the abstracts from the platform and poster session presentations at the 27th Annual Meeting of the Society of Environmental Toxicology and Chemistry Europe (SETAC Europe), held at Square conference centre, Brussels, Belgium, from 7–11 May 2017. The abstracts are reproduced as submitted by the author and accepted by the Scientific Committee. They appear in order of abstract code and alphabetical order per presentation type. The poster spotlight abstracts are included in the list of poster abstracts. The presenting author of each abstract is underlined.

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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY
In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, managers, engineers or others interested in environmental issues. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in North America in 1979 to fill the void, and quickly saw dynamic growth in the Society's membership, meeting attendance and publications.

A unique strength of SETAC is its commitment to balance the scientific interests of government, academia and business. The Society by-laws mandate equal representation from these three sectors for officers of the World Council and Geographic Unit Boards of Directors and Councils, and in the composition of committees and other society activities. The proportion of members from each of the three sectors has remained nearly equal over the years.

The Society is concerned about global environmental issues. Its members are committed to Environmental Quality Through Science®, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur. Therefore, SETAC publishes two globally esteemed scientific journals and convenes annual meetings around the world, showcasing cutting-edge science in poster and platform presentations. Because of its multidisciplinary approach, the scope of the science of SETAC is broader in concept and application than that of many other societies.

SETAC's growth is reflected in the founding of geographic units around the world. SETAC Europe was established in 1989 as an independent organisation, followed by SETAC Asia-Pacific in 1997 and SETAC Latin America in 1999. In 2002, the four existing organisations joined together under the governance of the SETAC World Council. SETAC Africa is the most recent geographic unit, which was adopted in 2012. As evidence of international acceptance of the SETAC model and of the great interest at the local level, regional chapters and branches have emerged in a number of countries.

SETAC publishes two journals: Environmental Toxicology and Chemistry (ET&C) and Integrated Environmental Assessment and Management (IEAM). Environmental Toxicology and Chemistry is dedicated to furthering scientific knowledge and disseminating information on environmental toxicology and chemistry, including the application of these sciences to risk assessment. Integrated Environmental Assessment and Management focuses on the application of science in environmental decision-making, regulation, and management, including aspects of policy and law, and the development of scientifically sound approaches to environmental problem solving. Together, these journals provide a forum for professionals in academia, business, government, and other segments of society involved in the use, protection, and management of the environment for the enhancement of ecological health and human welfare.

SETAC books provide timely in-depth reviews and critical appraisals on scientific subjects relevant to understanding a wide range of contemporary topics pertaining to the environment. These include any aspect of environmental chemistry, toxicology, risk assessment, risk management, or environmental policy.

SETAC has two administrative offices, in Pensacola, Florida, USA, established in 1992, and in Brussels, Belgium, established in 1993.

Keynote Abstracts

K1

Seas, Oceans, and Public Health in Europe

Lora Fleming[1], N. McDonough[2], M. White[1], O. McMeel[3], C. Domegan[3], S. Wujits[5], A. Blauw[6], J. Vera Prieto[7], M. Parga[8], J-B Calewaert[3], R. Garside[1], M. Garza[8], M. Depledge[1]/
[1]University of Exeter, UK; [2]European Marine Board, Belgium; [3]Seascape, UK; [4]National University of Ireland Galway, Ireland; [5]RIVM, Netherlands; [6]Deltares, Netherlands; [7]Travelecoology, Spain; [8]Submon, Spain

There is a growing body of evidence that the health of humans and the marine environment are inextricably linked. These linkages encompass both risks and benefits. On the risk side, natural events such as extreme weather and tsunamis can have devastating impacts on coastal populations, while pollution of the seas and global ocean by pathogens and toxic waste can cause illness and death. Conversely, the seas and global ocean can also be the source of essential ecosystem services and potential health benefits through the provision of healthy food, novel pharmaceuticals and related products derived from marine organisms, as well as through a contribution to general wellbeing from a close association with the coastal environment (i.e. the "Blue Gym" effect).
In the US, Oceans and Human Health (OHH) has been recognized as an important research and training area for more than 15 years. The OHH research programme in the US has provided a platform to bring together researchers from a diverse range of disciplines to address complex OHH questions and deliver societally relevant results, with continued funding. Europe has particular OHH needs and challenges. Surrounded by four seas and two ocean basins, and with a coastline of 89,000km, Europe is a truly maritime continent; approximately 43% of the EU population (over 218 million people) live in coastal regions, including in 194 coastal cities. To date, Europe has failed to develop a coherent Oceans and Human Health research collaboration. And on both sides of the Atlantic, there has been a failure to truly bring together the human health and marine communities to address the growing challenges of OHH. Hence, the European research community remains structurally unprepared to comprehensively respond to threats to human health and to optimize the potential for health and wellbeing benefits from our interactions with blue environments.

K2

10 years of REACH: achievements, scientific challenges and research needs

Geert Dancet, ECHA, Finland

This year's tenth anniversary of the European Chemicals Agency ECHA is an excellent opportunity to reflect on the main achievements of the REACH Regulation (Registration, Evaluation, Authorisation and Restriction of Chemicals). It is also timely to consider the scientific (environmental) challenges and research needs identified from working with this chemicals legislation. Several achievements of REACH will be highlighted in the presentation: increased availability of data, phasing out the most hazardous substances... Under REACH, industry is responsible for collecting information on the properties and uses of the substances they manufacture or import. New animal testing must only be done as a last resort and registrants can make use of alternatives methods and approaches. However such 'surrogate' data must be scientifically robust to be able to replace the standard in vivo tests that REACH prescribes. When examined many read-across and weight of evidence cases are inadequate, i.e. poorly documented &/or with inadequate scientific justification. This dilemma forms a challenge for regulatory science: regulators and the research community should work together to solve knowledge gaps and develop improved methods and approaches. One area to explore is to develop more integrated approaches between human health and environmental effects. For example mechanistic knowledge for human health effects could provide insight into mechanisms of action in ecotoxicology and could facilitate grouping and read-across approaches to predict properties of substances. Furthermore, 'new approaches' could be integrated with existing approaches: '-omics', systems biology, bioinformatics, standardised ecotox studies... are all valuable in themselves, but a coherent and integrated approach is needed to support efficient and effective regulatory decision making. Collaborative approaches between academia, regulators and industry can strengthen new developments and enhance their usability for reliable prediction of the long term effects of hazardous chemicals on human health and the environment and thereby contribute to the regulatory framework that can ensure safe use of chemicals.

K3

Product Environmental Footprint

Michele Galatola, European Commission - DG Environment, Belgium

The role of science in society is to help it to advance, in the sense of making the overall living condition of human society better, from the economic, social and environmental viewpoint. In most successful past civilisations, teachers and scientists were amongst the most respected professions in view of their key role for the continuation of our species.
It is possible to identify a cycle of science that usually starts with an important conceptualisation stage, followed by a trial & error stage, and ultimately – in the best cases – a deployment stage. Deployment is what mostly counts from the society perspective because it is when the large effects of any scientific novelty can really be experienced and have an impact on the society as a whole. However, for scientists, that is often the less interesting part of the "cycle" because there is no more room for testing, new theories and intellectual challenges, whilst it is all about "standardisation" of a method or procedure. Of course science continues, but once a certain approach is mainstreamed, inevitably it becomes less interesting from a scientific viewpoint.
This well-known dichotomy between scientific progress and its real-life impact is also valid for Life Cycle Assessment (LCA). We are now living the time when there may be the transition from the trial & error stage to the full deployment stage and the community is leaving this period with a tension between worry and excitement. The Life Cycle Assessment community has progressed about 50 years now and the method has been used for many industry in-house applications, but it has never been mainstreamed in policy making due to a number of limitations that will be discussed in this keynote. This situation might change in the coming months due to the finalisation of the largest trial & error experiment promoted in Europe by the European Commission and led by 24 industry sectors. The LCA-based Product Environmental Footprint (PEF) and Organisation Environmental Footprint (OEF) methods, developed by the Joint Research Centre of the European Commission, represent the best available practice of LCA and, in many respects, have introduced a Copernican revolution in this scientific community.
As all revolutions, the appearance of the PEF/OEF is facing ambivalent reactions within the scientific community and final users (industry, policy makers, consumer associations). The analysis of the reasons behind this and the implications of a wide deployment of PEF/OEF at European and international level, will be the focus of this speech.

Platform Abstracts

Multigenerational, epigenetic and evolutionary effects in human and environmental toxicology: from mechanisms to risk assessment

1
Genetics and evolution of tolerance to zinc pollution in the pseudometallophyte Arabidopsis halleri (Brassicaceae)
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Pollution can lead to environmental toxicity and threaten population maintenance. Population survival could imply the evolution of local adaptation for tolerance traits. Understanding which genetic diversity helps species to survive in polluted habitat is not onyl fundamental but could also help the development of new remediation strategies. Pseudometallophyte are plant species that occur on both metal-polluted and non-polluted areas (metallicolous, hereafter M, and non-metallicolous, hereafter NM, populations, respectively). They are therefore good models for the study of the genetics and evolution of metal-related adaptive traits, including metal tolerance (ability to survive and reproduced on metal-polluted soils). *Arabidposis halleri*, in particular, occurs in non-polluted areas but also colonized anthropogenic calamine (zinc (Zn) and cadmium (Cd)-rich) habitats. Although, its Zn tolerance was shown to be species-wide, quantities variation among populations supports a role in the adaptation to calamine habitats. The genetic basis of local adaptation to Zn pollution was investigated in *A. halleri* through several experimental strategies. First, the genetic architecture of Zn tolerance was analyzed comparing results from the QTL analysis of two interspecific crosses involving the nontolerant close relative *Arabidopsis lyrata* subsp. *petraea* and either a M or a NM accession of *A. halleri*. The subtractive comparison of obtained results suggested a role of some QTL regions in local adaptation. Those QTL regions are particularly known to include *A. halleri*-specific paralogs of the candidate gene MTP1 (METAL TOLERANT PROTEIN 1) encoding a Zn transporter involved in vacuolar sequestration. We therefore investigated the molecular evolution of MTP1 from a large set of *A. halleri* natural populations. Results confirmed that at least one paralog (AhMTP1-A2) could be involved in local adaptation to Zn-polluted habitats. In parallel, an third cross performed at the intraspecific level between a M and a NM accession of *A. halleri* was analyzed. We used 384 SNP markers issued from high-throughput sequencing of the genomes of parental plants for the genetic map construction. One major QTL was identified. It did not co-localize with QTL regions that have been identified so far at the inter-specific level. This suggests that the genetic diversity involved in local adaptation may differ among M population resulting from different events of colonization of Zn-polluted habitats.

2
The evolution of resistance to a gradient of contaminants in Gulf killifish (Fundulus grandis) populations from Galveston Bay, Texas, USA
E. Oziolor, Baylor University / Environmental Science; N. Reid, University of California Davis; S.L. Guberman, Baylor University / Department of Environmental Science; K. Young, Indiana University Bloomington; Z. Winfield, Baylor University; J. Apell, MIT / Civil & Environmental Engineering; L. Aguilar, Baylor University; P. Gschwend, MIT / Dept of Civil and Environmental Engineering; S. Usenko, Baylor University / Department of Environmental Science; J. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; A. Whitehead, University of California Davis / Environmental Toxicology; C.W. Matson, Baylor University / Environmental Science
The Houston Ship Channel (HSC) in Texas includes areas that have historically been found to contain substantial levels of mercury, dioxins, furans, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs). We collected *Fundulus grandis* from contaminated areas on the HSC, from sites expected to have intermediate contamination, and from reference sites to establish a total of 12 lab colonies. F1 embryos from HSC populations were up to 1,000x more resistant to PCB126- and 2-5x more resistant to coal tar-induced cardiovascular teratogenesis. We confirmed biparental inheritance and a genetic basis of protection through reciprocal crosses between a reference and contaminated population, and by conducting experiments on F2 embryos for select populations. Similar to patterns observed for *Fundulus heteroclitus* inhabiting polluted areas along the US Atlantic coast, HSC populations of *F. grandis* exhibit a reduction of both basal and induced cytochrome P450 1A (CYP1A) activity, suggesting a common mechanism of adaptation. We observe a gradient of adaptation that correlates with the level of pollution at multiple HSC sites. Thus, we sequenced 288 individuals from 7 populations to understand the genetic causes for this resistance. In addition, we look at contaminants in various environmental matrices to be able to understand the chemical drivers of the selective sweeps that we see in *F. grandis* populations. Such results suggest that the HSC can serve as a “natural laboratory” to study evolutionary processes driven by anthropogenic pollution.

3
Transgenerational effects and epigenetic inheritance following a chronic external gamma irradiation in Daphnia magna
M. Trijau, IRSN/PRP-ENV/LECO / PRP/ENV/SERIS/LECO; J. Asselman, Ghent University / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; O. Armant, C. Adam-Guillermin, IRSNPRPENVLECO / PRPENV/SERIS/LECO; K. De Schamphelaere, Ghent University (UGent) / Applied Ecology and Environmental Biology; F. Alonzo, IRSN
Aquatic and terrestrial ecosystems are exposed to radionuclides through planned discharges or accidental releases linked with the nuclear industry activities. Over the past decade, ecosystems protection against ionizing radiation has become a growing public, regulatory and scientific concern. Nowadays, ecological risk assessment for ionizing radiation is based on a majority of radiotoxocity tests under acute doses and short exposure time, far from realistic environmental conditions in which natural biota are actually exposed. Recently, studies in daphnids at chronic low doses have been conducted over several generations in order to understand the long-term effects of low doses of gamma radiation and explore the contribution of underlying molecular mechanisms, such as DNA alterations. In this context, radio-induced epigenetic modifications have never been investigated in invertebrate species although ionizing radiation were shown to induce changes in DNA methylation patterns and gene expression. This suggests that epigenetic modifications might be inherited across generations. In this context, the present study examines the potential transmission of epigenetic alterations caused by a life-long gamma irradiation to their non-irradiated progeny up to the third generation, using the microcrustacean *Daphnia magna* as a model organism. The aim of our study is to test whether effects on survival, growth and reproduction observed in daphnids exposed to gamma radiation (in generation referred as F0) might persist in their non-exposed offspring generations (referred as F1, F2 and F3) and whether it might be linked to modifications of DNA methylation patterns inherited across generations. Results showed significant effects of gamma radiation on fecundity only for the highest dose rate and no effect at organism level seemed to last across non-exposed generations. However, effects on DNA methylation at low dose rate and across generations are not excluded. Our study will give a better insight in the effect of ionizing radiation on epigenetic processes, their heritability across generation and their link to organism level effects.

4
Consequences of cadmium-induced epigenomes on mutation and animal fitness
J. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; C. Jackson, Indiana University / The School of Public and Environmental Affairs; N. Keith, S. Reynolds, S. Glaholt, Indiana University / School of Public and Environmental Affairs
There is a pressing need to understand how environmental conditions, including toxicant exposure, influence genome content, structure, and function and, in turn, how individuals and populations cope with changing environments. It is now understood that genomes are more than static, heritable, biological templates, but rather display a wide range of plasticity that is modulated by the environment. These environmental perturbations of the genome are functionally important as we are quickly learning that they contribute significantly to variation in individual physiologies, fitness, dynamics of populations, and influence adverse outcomes. Both base-substitution and structural mutations are known to contribute to genome plasticity. While mutation is a stochastic event, mutation hotspots exist both within and between genomes. The genetic events that give rise to mutation (e.g., altered DNA damage/repair pathways, miscued replication, recombination error, transposable elements) are beginning to be defined in terms of their functions, yet little is known of the mechanisms that destabilize DNA, potentiate mutation, and coordinate mutation within the genome. In this talk, we explore the hypothesis that environmental stress influences epigenetic states including histone modifications, which control access to the genome and affect organismal fitness by potentiating mutations. Understanding these processes will have profound implications for society and the long-term health of populations, which are living longer in the presence of a large and growing diversity of chemicals that can modify DNA.

5
Poster spotlight: MO001, MO002, MO003, MO004

Advances on the assessment of environmental pollutants to amphibians and reptiles

6
Bioaccumulation and tissue distribution of selenium in developing striped marsh frog tadpoles
C. Lanctot, Central Queensland University / School of Medical and Applied

Sciences; T. Cresswell, ANSTO Environmental Research / Institute for Environmental Research; P. Callaghan, ANSTO / Life Sciences; S.D. Melvin, Griffith University / Australian Rivers Institute
Selenium (Se) is a nutritionally essential element that occurs naturally and ubiquitously in the environment in both organic and inorganic forms. Despite being essential for animal health and fitness, Se has a relatively narrow range between deficiency and toxicity, and excess Se can cause a variety of adverse effects in aquatic organisms. Selenium may pose serious long-term threats to aquatic ecosystems because it has the capacity to bioaccumulate and biomagnify in the food chain. Amphibians may be particularly vulnerable to contaminants during their larval aquatic life stage, because they can accumulate toxic ions through their skin, gills, lungs and digestive tract. Few attempts have been made to understand the tissue-specific uptake of trace elements, and the impacts of chemical forms, in larval amphibians undergoing metamorphosis. We used radiolabelled ^{75}Se to explore the biokinetics and tissue distributions of the two dominant dissolved forms that occur in surface waters, selenite (SeIV, SeO_3^{2-}) and selenate (SeVI, SeO_4^{2-}). Tadpoles of the native Australian frog *Limnodynastes peronii* were exposed to ^{75}Se in both forms, and gamma spectroscopy was used to track their uptake and retention over time. Tissue-specific kinetics and biodistribution of Se were quantified throughout metamorphosis using individually dissected organs and whole-body autoradiography. Results showed that the bioconcentration of SeIV to be 3 times greater compared to SeVI, but rates of elimination were similar for both forms. This suggests a change of Se speciation within the organism prior to excretion. Depuration kinetics in whole body and individual organs were best described by a one-phase exponential decay model. Tadpoles retained approximately 20% of the accumulated Se after 10 to 27 days of depuration in clean water. Selenium bioaccumulation was greatest in digestive and excretory organs, as well as the eye lens, which may directly relate to previously reported Se-induced impairments. Results demonstrate how the use of radiotracing techniques can significantly improve our understanding of trace element toxicokinetics and tissue distributions, during amphibian development. Knowledge of the distribution and speciation is essential to understanding the risks associated with Se exposure, but more research is needed to understand the fate and metabolism of Se in various tissues.

7

Common toad (Bufo bufo) and common frog (Rana temporaria) in agricultural landscapes in Germany - results of a monitoring study

F. Groezinger, G. Schmidt, BASF SE; G. Montinaro, J. Ludwigs, Rifcon GmbH; P. Dohmen, BASF SE / Landw Versuchsstation APDRO
Field data about the behaviour and prevalence of amphibians in agricultural fields is rather scarce. Therefore, we have conducted monitoring studies of the common toad and common frog in an agricultural landscape in southern Germany. Using a combination of drift fences and pitfall traps, spring migration timing and presence of the two species were recorded in agricultural fields for two consecutive years. Further, methods for individual identification, to assess activity patterns and the use of telemetry as a suitable method for field trials was investigated. The spring migration of adult common frogs and common toads to and away from their breeding pond occurred in March and early April. This movement through agricultural landscapes corresponds to the early growth stages of cereals in this region. Metamorphs were also captured emigrating from the pond starting in June, and thus at later stages of crop development. These data contribute to the knowledge about amphibian phenology and give further information about suitable methods to investigate their presence and behaviour in agricultural landscapes for assessing potential impacts of agricultural measures.

8

Toxicity of chemicals to amphibians and reptiles. Assessment factors and comparative sensitivity

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Amphibians and reptiles have not been traditionally considered in environmental risk assessments of pesticides, which has generated some debate about whether risk posed by pesticides on these animals are covered by surrogate taxa. In order to develop a scientific sound and robust risk assessment scheme it is necessary to have enough information on the biological relevance of effects observed in laboratory studies in view of population level effects, to identify sensitive life stages and to compare sensitivity of our target study groups with that of their surrogates. With these purposes, a systematic review of toxicological literature on amphibians and reptiles was conducted. After retrieval from different sources and filtering of references, 4504 records were screened and proposed for data extraction. Informartion on species, age, sex, chemical substance, exposure route and duration, type of recorded endpoint, type of response, exposure concentration, mean effect value of the control and exposed groups and the reported variability measures of these mean effects, and statistical significance of the comparison was extracted from each valid reference. To estimate extrapolation factors from lab to field data, identify senstive stages and compare among taxa, we used LC/LD/EC/ED/NOEC values when possible, calculating Hedge's d values as the metric of standardized effect size in the rest of cases. The results of the literature review are currently being processed and will be included in the final presentation. Financed through the EFSA procurement OC/EFSA/PRAS/2015/01.

9

Coverage of Amphibians and Reptiles by the Pesticide Risk Assessment for Birds and Mammals

F. Streissl, EFSA / Pesticides Unit; P. Berny, VETAGRO-SUP / Toxicology; M. Ortiz Santaliestra, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; L. Petschick, University of Koblenz Landau / Institute of Environmental Sciences; S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products
Vertebrate biodiversity is in decline with amphibian and reptile species being more threatened and experiencing faster decline rates than birds and mammals [1] [2] [3]. Xenobiotic chemicals including pesticides are hypothesized as being one of the causes of the decline of amphibans and reptiles. There are indications that the current risk assessment schemes may not be protective for amphibians and reptiles. In order to close this potential gap in the pesticide risk assessment, the European Food Safety Authority (EFSA) was tasked to develop a risk assessment scheme for amphibians and reptiles exposed to Plant Protection Products (PPP) after intended uses. The aim of the study was to investigate the coverage of the exposure of amphibians and reptiles by exposure estimates for birds and mammals and to identify potential differences in the relative importance of different exposure routes among amphibian and reptile groups. Worst case exposure calculations were conducted in order to compare the relative importance of oral and dermal exposure pathways, to identify the groups of amphibians and reptiles with the greatest oral and dermal exposure and to investigate whether the exposure assessment in the first tier risk assessment for birds and mammals cover amphibians and reptiles (only for oral exposure). Preliminary results indicate that oral exposure of birds may cover amphibians. Dermal exposure of lizards and snakes is of high importance and in the same range as the daily dietary exposure of birds. Comparison of daily dietary exposure and dermal exposure give an indication that both exposure pathways are of high importance and both need to be considered in the risk assessment for amphibians and reptiles.

10

Risk assessment proposal for plant protection products for terrestrial life stages of amphibians

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Amphibians can be found in agricultural landscapes and as a result may be exposed to plant protection products. While aquatic life-stages are considered to be covered by the standard data requirements for aquatic organisms (especially fish), the situation is less clear for the amphibian terrestrial life-stages. In this contribution, a first proposal is presented on how to conduct risk assessment for plant protection products and terrestrial life-stages of amphibians. It briefly discusses available evidence and considers aspects of amphibian biology, exposure and toxicity. Special emphasis is put on avoiding unnecessary vertebrate testing by exposure driven approaches and also by making use of existing vertebrate toxicity data that could be used as surrogates for amphibians. Options for toxicity testing are presented as a tiered approach, progressing from simple worst case laboratory testing, via semi-field enclosures to ultimately full scale field testing and monitoring. Proposals are made for triggers that help decide on the necessity to progress to higher tiers. Finally, remaining uncertainties and research items are considered by proposing a way forward (road map) for generating additional data to inform amphibian terrestrial risk assessment.

Combined effects of chemical and environmental stressors: from local stressors towards climate change (I)

11

Unravelling the effects of multiple stressors on the aquatic invertebrate community in the region of Madrid (Central Spain)

A. Rico, IMDEA Water Institute / Aquatic Ecotoxicology; C. Alonso, P. García, A. Romero, A. Castaño, L. Nozal, IMDEA Water Institute; M. Vighi, IMDEA Water Institute / Earth and Environmental Sciences; A.A. Sanchez, IMDEA Water Institute / Aquatic Ecotoxicology
Combined effects of chemical pollution and water scarcity are one of the most important threats to aquatic ecosystems in semi-arid regions. The low dilution potential in aquatic ecosystems receiving waste water can enhance chemical exposure, leading to devastating direct and indirect toxic effects. The aim of this study was to evaluate the combined effects of multiple stressors on aquatic ecosystems in scenarios of water scarcity that are characteristic of (semi-)arid regions. In particular, this study focused on identifying the main stress factors that are influencing aquatic communities in the (semi-)arid region of Madrid (central Spain) and on describing the most relevant traits characterising adaptation to the

combined effects of pollution and water scarcity. Sixteen sites were selected in the watersheed of the Tagus river and sampled in three seasonal periods (spring, summer, fall). The selection was based on a water quality and flow variability gradient. The basic physico-chemical water characteristics were measured, together with inorganic (metals) and organic microcontaminants in water and sediments. The macrozoobenthos community was sampled by using a Surber net and pebble traps maintained in situ for two weeks to allow colonisation. Daily-based hydrological data were additionally obtained. The complete examination and elaboration of the data is still ongoing. Preliminary results indicate that the structure of the macroinvertebrate community is extremely different in the various sites. As expected, good quality sites are characterised by more sensitive taxa (such as Plecoptera, Ephemeroptera, Tricoptera), while resistant taxa (e.g. Chironomidae, Oligochaeta, Culicidae) are typical for bad quality sites. The results of a screening analysis indicates that the number of compounds that seems to be present in the bad quality sites is higher than in the less impacted sites and shows a trend towards a higher number of compounds appearing in the spring campaign as compared to the summer campaign, with more than 100 compounds being characterized (pharmaceuticals, pesticides, illicit drugs). Through an in-depth analysis of pollution data together with water flow variability and related physico-chemical parameters, the most important traits, capable to characterise taxa better adapted to combined conditions of pollution and water scarcity, will be identified.

12
A causal assessment approach that rapidly evaluates multiple stressors in stream systems
J.M. Diamond, Tetra Tech, Inc.; A. Roseberry-Lincoln, C. Boschen, Tetra Tech; R. Kolb, City of San Diego
Benthic macroinvertebrate assessments are frequently used by environmental resource agencies to determine whether aquatic life condition is unharmed. If a site is assessed as impaired a causal assessment is needed to determine why the biota are impaired. Such an assessment involves a weight of evidence approach that frequently considers multiple stressors and multiple lines of evidence. We recently developed a novel screening approach that uses several abiotic factors in a cluster and principal components framework to identify comparator sites appropriate to the test site or stream reach and indicate the stressors most likely to be responsible for observed impairment. Additional tools within this approach, including taxa tolerance values to specific stressors such as dissolved solids, species sensitivity distributions for specific chemicals, and GIS-based models that identify hydrologically or physically modified channels, are used to further diagnose the cause of observed impairment. Weight of evidence is used to present the results and inform the user of the likely cause(s) as well as uncertainties due to critical gaps in stressor information. The approach is built based on benthic macroinvertebrate taxa information and algal (periphyton) information is currently being included as well. It is expected that greater diagnostic capabilities will be obtained with the inclusion of data from both assemblages. The approach has been tested at several types of sites in southern California and can be readily transferred to other regions.

13
Predicting the synergy of multiple stress effects
M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology; K. Foit, UFZ - Helmholtz Centre for Environmental Research; S. Knillmann, Helmholtz-Centre for Environmental Research (UFZ) / Department of System Ecotoxicology; R. Schaefer, University Koblenz Landau / Institute for Environmental Sciences; H. Liess, University of the Bundeswehr München
Toxicants and other, non-chemical environmental stressors contribute to the global biodiversity crisis. Examples include the loss of bees and the reduction of aquatic biodiversity. Although non-compliance with regulations might be contributing, the widespread existence of these impacts suggests that for example the current approach of pesticide risk assessment fails to protect biodiversity when multiple stressors concurrently affect organisms. To quantify such multiple stress effects, we analysed all applicable aquatic studies and found that the presence of environmental stressors increases individual sensitivity to toxicants (pesticides, trace metals) by a factor of up to 100. To predict this dependence, we developed the “Stress Addition Model” (SAM). With the SAM, we assume that each individual has a general stress capacity towards all types of specific stress that should not be exhausted. Experimental stress levels are transferred into general stress levels of the SAM using the stress-related mortality as a common link. These general stress levels of independent stressors are additive, with the sum determining the total stress exerted on a population. With this approach, we provide a tool that quantitatively predicts the highly synergistic direct effects of independent stressor combinations.

14
Combined environmental and chemical stress: impact on bioenergetics fluxes
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The lack of ecological realism in current prospective environmental risk assessment (ERA) is widely recognised as a limitation in this field. Since organisms live in a multistressed environment, involving both chemical and environmental stressors, it is important to understand how these combined stressors can affect individuals and subsequently their populations. One way of including more ecological relevance in ERAs is the use of environmental scenarios that represent variation in environmental factors such as food, temperature, predation, competition and in factors that influence exposure. All these factors will influence the capability of an organism to grow and reproduce as well as its resilience to additional stressors. Since growth and reproduction are driven by an organisms' energy balance, Dynamic Energy Budget models are particularly well suited to integrate toxicant and environmental stressors. Indeed, the DEB theory analyses the fluxes of energy within an individual, how stressors can impact these fluxes, and how this will affect the organism's life history traits. This mechanistic description of an individual can then be used as a building block for a population model. In this project, we performed a meta-analysis of the literature in order to analyse the effects of combinations of ecological and chemical stressors on individuals. We then used this analysis to derive quantitative relationships that describe the effects on their bioenergetic fluxes. We analysed how these stressor combinations affected the life-history traits of the organisms and their ability to cope. We assessed the implementation of these patterns using a DEB based model for *Ceriodaphnia dubia*. Using the derived quantitative relationships, we predicted the effects of mixtures of environmental and/or chemical stressors on the growth, the reproduction and the survival of individuals. This mechanistic description of the organisms can then be used as a building block in order to assess the effects of these mixtures on a higher level of organisation, through a population model for instance. The accuracy of our predictions was evaluated by comparison with experimental data. The resulting models will have the potential to account for the effect of chemicals on individuals and populations in different environmental scenarios.

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Fate and effect of sediment-associated triclosan: Impact of T. tubifex, sediment characteristics, and temperature
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Aquatic environments are a likely recipient for household and personal care products (HPCs) through ‘down-the-drain’ routes in urbanized areas. Due to the physical/chemical properties of HPC ingredients, such as triclosan (TCS), these chemicals will accumulate in sediments and provide a potential threat to benthic organisms. This study examine TCS uptake and elimination rates in the deposit-feeding oligochaete *Tubifex tubifex* exposed to TCS either in water or in sediment under varying conditions representing temperate and subtropical climates. Specifically, we test the impact of temperature (17 and 28?C) and sediment characteristics (i.e., grain size and total organic matter (TOM)) Sediment for the experiment was collected from a temparate and subtropical area located away from point sources; Holbæk fjord in Denmark and by the mountains in The Pearl River in China. The sediment was sieved (< 125μm) and subsequently characterized, prior to TCS spiking. Two sets of experiments were conducted to estimate uptake and elimination rates in worms: *Uptake experiment*: Dietary and waterborne uptake was investigated based on 4 hour exposure to six different TCS concentrations. *Elimination experiment*: *T. tubifex* was first exposed to TCS contaminated sediment or -water for 10 days. The uptake phase was followed by an 11 d elimination phase in clean water or sediment. The experiment was terminated at day 1, 2, 4, 7, 11 (n=3). TCS uptake from water was neglible, whereas uptake from both sediments (i.e., China, Denmark) was significant. Worms did not eliminate TCS once taken up regardless of treatment. The uptake rate was dependent on sediment characteristics (higher uptake rate in sediment with smaller grain size and higher TOM (i.e., CH sediment)). The effect of temperature was non-significant suggesting that spatial differences will be caused primarily by sediment characteristics. Dissipation of TCS from sediment was 5-30% higher in the presence of *T. tubifex* than without worm presence suggesting at least two possible explanations; 1) *T. tubifex* remove sediment-associated TCS by accumulation and potentially by biotransformation, and B) the presence of *T. tubifex* stimulate microbial degradation. Further analyses include TCS elimination and uptake from water which will provide further insights into the fate of TCS in the aquatic environment, and the effect of *T. tubifex*.

In situ measurement of nanoparticles

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Single Cell ICP-MS: quantifying exposure and dose of gold and silver NPs to freshwater algae
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Recently the production and use of engineered nanomaterials (ENMs) have experienced a drastic increase in manufacturing and use in commercial products. This increase in use results in a significant risk of their release into the environment and their interaction with aquatic species such as algae or fish. The measurement of exposure (amount of contaminant in the water), dose (amount of contaminant taken up by organism) and response has been difficult to quantify due to the limitations of being able to measure the amount of metal within a single cell. Single cell ICP-MS

(SC-ICP-MS) is a new ICP-MS technique based on single particle (SP)-ICP-MS, which allows for the measurement of the metal content of individual single celled organisms. SC-ICP-MS allows for the measurement of exposure and dose to single celled organisms in both environmental and human health studies with minimal sample preparation and at relevant contaminant concentrations. SC-ICP-MS allows for the quantification of both metal concentration within the cell and the number of cells containing that metal. It permits for the measurement of intrinsic metal as well as the uptake of both dissolved and nanoparticulate (NP) metallic contaminants. Here we examine the uptake and depuration of both dissolved (Au) and NP (Au and Ag) to freshwater algae. The algae were exposed to Au and Ag nanoparticles over a period of four days. The amount of NPs in suspension (exposure) and the amount of metal taken up by the cells (dose) were measured by SC-ICP-MS and the toxicity (biological response) was measured at various time points. We show that the percentage of the cell population containing metal NPs increases with both time and initial exposure concentration. The uptake of multiple NPs into individual cells was observed for higher concentration exposures along with evidence for intercellular interactions with the NPs.

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Predictability of silver nanoparticle speciation and toxicity in ecotoxicological media

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The use of silver nanoparticles (AgNP) has increased significantly over the past decade due to their antimicrobial effect. Since the antimicrobial effect of AgNP is mediated by the release of silver ions (Ag^+), it was the purpose of this study to investigate the silver release of silver nanoparticles dispersed in a wide variety of (eco-)toxicological test media for aquatic organisms (algae, plants and crustaceans), terrestrial organisms (bacteria) and cells, to understand the contribution of the chemistry of the surrounding environment to the bioavailability and hence to the toxicity of AgNPs. It was the second aim to predict silver speciation in aqueous solutions by equilibrium speciation calculation and validate the results by comparison with experimental speciation using ultracentrifugation and membrane filtration. Silver amounts were quantified using GF-AAS, ICP-OES/MS and UV/VIS. The release experiments were conducted in the test media without organisms in the time frame of the test periods. The dissolved silver concentrations were controlled by the fast initial release of a limited amount of Ag^+. After this initial release, the media components chloride and proteins were controlling the available dissolved silver by precipitation and complexation. Further release of silver ions due to oxidation was not observed in the time frame of our experiments, except for media with very high chloride content. The findings revealed that theoretical prediction of bioavailability of ions released from redox-active nanoparticles can be done without including kinetic information, when experimental speciation data of the initial conditions are available and the kinetics of ion release is slow compared to the time frame of the toxicological tests. This approach could circumvent the challenging intricacy of kinetic modelling in complex biological media for these kind of nanoparticles.

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Degradation kinetics of C60 fullerene aggregates suspended and aged under realistic environmental conditions

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Fullerenes are carbon-based nanomaterial composed by sp^2-hybridized atoms organized in a hollow polyhedron shape. These carbon allotropes are emitted as by-products in highly energetic processes of natural origin (such as volcanism, forest fires, meteorite impacts,…) or anthropogenic sources (combustion sources, vehicle exhaust and brakes, some personal-care products, etc.). Recent studies have detected fullerenes in several environmental compartments including the atmosphere, soils and sediment, and river water. However, little is known about the degradability and transformation products of fullerenes and, more precisely, about the degradation products of C_{60} fullerene, the most ubiquitous in the environment. In water suspension, the degradation of fullerene aggregates is expected to happen both physically (changing the size and shape of aggregates) and chemically (functionalizing fullerenes surface) and these processes would be strongly ruled by media parameters, hence the importance of conducting degradation experiments in conditions that simulate real environmental scenarios. The lack of sensitive methods for the analysis of fullerenes and their derivatives has hampered the search of fullerene degradation products in the environment. In the present work, a method has been developed for the analysis of fullerene degradation products, including hydroxylated fullerenes (fullerenols), oxidized products (epoxides and carbonyls) and fullerene dimers C_{60} fullerene was suspended in an environmentally relevant manner and aged in different artificial waters simulating relevant conditions in terms of pH, salinity and humic substances content. Samples were extracted after several time of simulated sunlight exposure and the kinetic of degradation were studied. Overall C_{60} was observed to be a rather persistent compound, although different degradation products (mainly epoxides and dimers) were identified and semi-quantified. The pH, salinity and humic substance content had a significant impact in the degradation of this fullerene and the presence of open-cage compounds was also assessed. *Acknowledgement - This work was supported by the Spanish Ministry of Economy and Competitiveness through the projects INTEGRACOAST (CGL-2014-56530-C4-1-R) and NANO-transfer (ERA_NET SIINN PCIN-2015-182-CO2-01) and UK National Environmental Research Council through the project Trojan Horses (NE/L006782/1).*

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Flows of engineered nanomaterials through waste treatment to the environment

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Background: Although engineered nanomaterials (ENMs) are present in a growing number of consumer products, their potential impacts on human and environmental health are still poorly understood. More specifically, the knowledge about the releases of ENMs to the environment is associated with large uncertainties, which hinders the accurate modelling of their potential impacts and risks to the environment. The aim of this study is to improve the quantification of the flows of ENMs from the use phase through waste treatment to the environmental compartments. Materials and methods: This work relies on probabilistic material flow analysis (PMFA) to assess the flows of nano-TiO_2, nano-ZnO, nano-CuO, nano-Ag, nano-SiO_2 and carbon nanotubes (CNT) from their production, manufacturing and consumption through waste treatment to the environment. This assessment is performed at the national scale for all European Member States, Norway and Switzerland. The first step of this work is to improve the precision of the ENMs allocation to the product categories, based on online consumer product inventories, commercial platforms and Google searches. Once the distribution of ENMs in the different types of applications was assessed, their flows towards the waste treatment processes (landfilling, incineration, recycling and reuse) could be quantified based on reports from government organizations or when these were not available, on the Eurostat database. A qualitative assessment of the data reliability is performed on the references used for each country. Finally, the potential elimination of ENMs, their release to the environment or their flow back to production, manufacturing and consumption are assessed based on literature and expert elicitation. Results: The flows entering the waste treatment processes show significant variations from one country to another, confirming that the European (continental) scale is not sufficient for this assessment. The most relevant waste treatment process regarding the overall potential releases appears to be recycling. Conclusions: To the best of our knowledge, this work constitutes the first quantitative assessment of flows of ENMs to waste treatment processes in all European countries. It forms an important piece in the discussion on the relevance of the end-of-life stage on releases of ENM and also on the potential of ENMs to contaminate waste flows.

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Modelling nanoparticle fate in wastewater treatment plants

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Engineered nanoparticles (ENP) have been the subject of hazard investigations the past decades, but prediction of their fate has been less well developed, relying mostly on transfer factors. ENPs in wastewater treatment plants (WWTPs) are usually assumed to be ca. 95 % towards sludge irrespective of ENP and WWTP properties, but a high variability in transfer factors is found instead. The attachment kinetics of nominally 28 nm citrate coated, 80 nm citrate coated and 8o nm PEG coated silver ENP (Ag NP) to WWTP biosolids and their subsequent sedimentation simuating a clarifier was investigated, using biosolids form a WWTP in Gothenburg, Sweden and varying physical paramters as well such as shear, and total suspended solids concentration (TSS). The goal was to provide insights in ENP and WWTP specific paramters explaining the variation in ENP removal in WWTPs. Larger Ag NP were found to attach faster to WWTP biosolids comapred to larger ones, coatig having no effect. Increasing shear rate had little effect. The concentration of the 28 nm AgNP was thus relatively larger during sedimentation experiments suggesting that relatively smaller ENPs are more likely to leave the WWTP with the effluent. Physical paramters such as shear had little effect and while increasing TSS enhanced attachment rates, it also slowed down sedimentation rates. The present results, combined with literature data is used to construct a WWTP and ENP specific model explaining some of the variation in transfer of ENPs during transport through WWTP.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (I)

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A global overview on exposure levels and biological effects of trace elements in penguins

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Trace elements are chemical contaminants that can be present in almost anywhere on the planet. The study of trace elements trace in biotic matrices is a topic of great relevance for the implication that can have on wildlife and human health. Penguins are very useful, since they live exclusively in the Southern Hemisphere and represent about 90% of the biomass of birds of the Southern Ocean. The levels of trace elements (dry weight) in different biotic matrices of penguins were reviewed here. Maps of trace element records in penguins were included. Data on exposure and effects of the elements trace in penguins were collected from the literature. The most reported trace elements in penguins are aluminum, arsenic, cadmium, lead, mercury, copper, zinc and manganese. Trace elements have been measured in 11 of the 18 species of penguins that exist. The most studied biotic matrices are feathers and excreta. Most of the studies have been performed in Antarctica and sub-Antarctic Islands. Little is known about the interaction among metals, which could conduct to a better knowledge about certain mechanisms of detoxification in penguins. Future studies on trace elements in penguins must incorporate other metals such as vanadium, cobalt, nickel and chromium. Data of metals in the species *Eudyptes pachyrhynchus, Eudyptes moseleyi, Eudyptes sclateri, Eudyptes robustus, Eudyptes schlegeli, Spheniscus demersus, Spheniscus mendiculus and Megadyptes antipodes* are urged. It is quite needed to correlate levels of metals in different biotic matrices with the effects on different species and geographical locations. *Acknowledgement* - Winfred E. Espejo is a graduate student at the Universidad de Concepcion, Chile, who is sponsored by the CONICYT-Chile for PhD studies. This study was financially supported by the project INACH RG 09-14, T12-13 and INACH T31-11. Thanks also are given to project 214.074.051-1.0 and 216.153.025-1.0 of the Research Division of the Universidad de Concepción.

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Opposing gradients for undesirable and essential elements in saithe from Skagerrak to Barents Sea - What goes around does not always come around?

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This baseline studies provides comprehensive information about the content and distribution of undesirable and essential elements in saithe (*Pollachius virens*) from Norwegian waters. 1620 saithes were sampled from Skagerrak, the North Sea, the Norwegian Sea and the Barents Sea in the years 2010 to 2013. We have quantified arsenic, heavy metals, and some mineral and essential trace elements in muscle and liver, mapped the results geographically and explored the dataset. The concentrations of arsenic, cadmium, lead and mercury were low compared to levels from cod and Greenland halibut measured previously and clearly below EU food safety maximum levels. In saithe filet, the concentrations of arsenic and mercury formed a geographical gradient with the highest values in the south, decreasing towards the north, while cobalt, copper, iron, manganese, selenium and zinc had the opposite trend. In liver, all elements displayed decreasing levels towards the North. All trends are only present in smaller saithes, except for mercury where the trend was clear for all sizes. Some of the elements had been quantified in sediment samples throughout the study area by the Mareano effort. Interestingly, mercury, but also copper and zinc displayed decreasing concentrations from south to north in sediments, the same trend as in filet for mercury, but the opposite trend as for copper and zinc.

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Assessment of Exposure to Heavy Metals via ingestion, inhalation and dermal contact to Urban and Background Soils in Turkey

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Heavy metals accumulate in soil and pose negative effects on living organisms and human. In this study, soil samples from urban and background sites located in Aksaray, Usak, Konya, Elaz?g, Malatya, Kayseri, Mersin, K?r?kkale, Kars, Kastamonu, Çank?r?, Izmir, Istanbul, Antaya and Van provinces of Turkey were analyzed to investigate the concentrations of Cd, Hg, Pb, V, Cr, Mn, Co, Ni, Cu, Zn and As using ICP-MS instrument. Based on the results obtained from the analysis, ecological risk assessment and human exposure calculations were conducted. Concentrations of Cd, Hg, Pb, V, Cr, Mn, Co, Ni, Cu, Zn, As in urban areas were 379, 194, 18034, 32294, 41890, 205343, 5957, 1621, 13215, 34035, 5649 μg/kg, respectively whereas it was 255, 52, 9621, 18363, 40202, 159140, 5444, 577, 6642, 18023, 3821 μg/kg, respectively in bakcground sites. It was determined that only the concentration of As was exceeded the limit values set by Legislation on Soil Pollution Control and Areas contaminated by Point Sources in Turkey. Carcinogenic risk values was detected as 4.71×10^{-3} and 1.81×10^{-3} in urban areas for children and adults, respectively while it was 3.56×10^{-3} and 1.37×10^{-3} at background sites, respectively. Non-cancerogenic risk values were 304.9×10^{-3} and 30.7×10^{-3} in urban areas for children and adults respectively while it was 252.4×10^{-3} and 25.8×10^{-3} at background sites, respectively. Results showed that Van and Aksaray had the highest ecological risk whereas Istanbul showed the high ecological risk level.

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Predicting Arsenic Bioavailability in Contaminated Soils by Using In Vitro Gastrointestinal Bioaccessibility and Speciation for Site-Specific Risk Assessment

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Exposure risk associated with soils contaminated with As is assessed by human health risk assessment (HHRA). Extensive research efforts have been directed toward development and application of in vitro gastrointestinal methods to predict relative bioavailable As (RBA As) across four continents. RBA As vs. IVBA As regression equations are used to predict RBA from IVBA. These methods are gaining regulatory acceptance for HHRA on contaminated sites. However, the ability of bioaccessibility methods to predict RBA As for contaminated sites and sources outside those used in developing the IVIVC regression equation is unknown. The objective of the current study is to evaluate the ability of several international bioaccessibility methods to predict RBA As for 12 contaminated sites. Arsenic bioaccessibility, determined using five in vitro methods, ranged from < 1 to >90% for 27 arsenic contaminated soils. Bioaccessible As (IVBA As) was determined by several in vitro gastro(intestinal) methods including the SBRC, RBALP, USEPA Method 9200, OSU IVG, and the California Bioaccessibility method (CAB). Relative bioavailable (RBA) As was determined from dosing trials using juvenile swine. Regression equations from published IVIVC were used to predict RBA As from in vitro arsenic bioaccessibility. In general, the IVBA methods used in this study met criteria for predicting RBA As. Predicted RBA As followed the trend CAB ≥ OSU IVG > RBALP, SBRC, USEPA 9200. The most accurate predicted RBA values fall within the 90% RBA As confidence interval (C.I.). However, conservative values that exceed the 90% C.I. are desirable because they are protective from a regulatory perspective. Several in vitro methods meet the criteria where predicted RBA As ≥ measured RBA As can be used for site specific HHRA. Results from this study show IVBA methods provide reliable estimates of RBA As for use by contaminated site managers. However, several of the IVBA methods underpredicted RBA As when applied to specific sites. Arsenic speciation alone is not predictive of IVBA or RBA As. However, As speciation is very important to provide information on IVBA or RBA As results. Supplemental site data (i.e., arsenic speciation) may be required for proper selection of methods by risk assessors to accurately predict RBA As and human exposure and gain acceptance by the regulatory community.

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Assessing the human bioaccessibility of automotive exhaust catalysts in urban road dust

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Road dust enriched with toxic metals and metalloids is a potential contributor to urban air pollution; thereby, posing a potential human health risk. While the total concentrations of many metals in road dust have been well characterized to date, limited data exists on the elemental bioaccessibility of road dust particle size fractions capable of being inhaled or respired. Most notably, there is little data on emerging metals of concern such as platinum group elements (PGE). The goal of this study is to examine the physical and chemical bioaccessibility of Canada's Chemicals Management Plan priority metals in road dust. Road dust samples (n=65) were collected in 2015 and 2016 in cooperation with the City of Toronto. The city uses regenerative sweepers to clean its streets, which dry-fractionate collected material into coarse and fine fractions. The particle size distribution of fine dust sweeps of human health concern were analyzed using a laser diffraction particle size analyzer. Total PGE concentrations were measured in dust samples using NiS fire-assay-INAA. The lung simulant, Gamble's solution, was used to extract the bioaccessible fractions of PGE. Metal concentrations were measured in extracts with ICP-MS. The bulk of particles (50th percentile) in fine sweeps had a diameter which ranged between 6.5 and 14.6 microns. PGE measurements confirm an enrichment of Pd, Pt, Rh and Ir in road dust, attributed to exhaust emissions. Pd was observed to occur at the highest relative concentrations in fine road dust, with a City median of 132 μg/kg, followed by Pt (median: 50 μg/kg) and Rh (median: 19 μg/kg) and Ir (median: 0.35 μg/kg), while Os and Ru were below limits of detection and/or limits of quantification. Fine/coarse enrichment ratios for PGE increased from lowest to highest in the following order: Pd(2.5)< Rh(2.6)< Pt(2.8)< Ir(7). The observed enrichment of PGE in the finer fractions of road dust is of concern, given the greater relevance of the fine fraction to environmental and human health. In

Gamble's solution, Pt was determined to have the highest solubility (mean: 66%). This was followed by Rh (mean: 28%) and Pd (mean: 5%). The results show that a fraction of PGE are likely present as soluble species under ambient conditions, despite their application in elemental form as catalysts. This study highlights a need to conduct testing using field-collected samples to understand the physical and chemical bioaccessibility and toxic potential of PGE.

Poly- and perfluoroalkyl substances (PFASs): Recent developments, sources, transport, fate and toxicity (I)

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Assessment of PFAS in soil and groundwater: new analytical technologies for comprehensive analysis of PFAS including precursors

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Poly- and perfluoroalkyl substances (PFAS), including perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are commonly elevated in soil and groundwater associated with the use of aqueous film-forming foams (AFFFs). Polyfluorinated compounds, in addition to many other perfluorinated compounds (collectively termed precursors), are biotransformed in the environment to form simpler perfluorinated carboxylates and sulphonates (such as PFOS and PFOA), which are persistent in the environment as they are not susceptible to further biodegradation. Consequently, in addition to PFOS and PFOA, there are numerous other "precursor" compounds in AFFF which present an ongoing source of PFOS and PFOA and other perfluorinated sulphonates and carboxylates. PFAS contaminated source zones are often associated with large plumes as the majority of PFAS are anionic and are not retarded significantly in aquifers, however some precursors are cationic and will bind to soils via ion exchange mechanisms, producing a lesser mobile source mass. The precursors are not accounted for by the US EPA analytical method 537 (LC-MS/MS). Additional analytical challenges are associated with the required low reporting/detection limits to be consistent with the US EPA drinking water provisional health advisory of 0.2 μg/L and 0.4 μg/L for PFOS and PFOA, respectively. This presentation will describe new analytical methods developed to quantify the total concentration of precursors and PFAS in water and soil samples.

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Determination of precursors of perfluoroalkyl acids in surface and wastewaters: application to some case studies

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The identification and the quantification of PFAA precursors is a key issue in their risk assessment but it is difficult and problematic for the lack of analytical standards or the challenges associated with available analytical techniques. Limits of detection of the target methods in the most of the cases are too high to be applied to field samples, unless for specific cases of industrial pollution. In order to assess the total amount of "potential" PFAA (already present in the aqueous sample and that can be formed by oxidation) a method for the quantification of the total amount of PFAA precursors in natural waters, after a persulphate oxidation of the sample, has been tested and validated. The method, modified from a literature one, has been applied on surface waters and treated wastewaters both in field and pilot scale tests. The compatibility of the literature method with the on-line-SPE-UHPLC-MS/MS method has been evaluated by studying the matrix effects and method repeatability. Furthermore conversion efficiency of selected precursors and stability of main perfluoroalkyl acids under the oxidation conditions have been evaluated in order to assess its applicability in experimental testing of treatment technologies and even in field studies. The method has been successfully applied to estimate the effect of the passage through the soil of wastewaters in managed aquifer practices including indirect potable reuse with the augmentation of natural resource (i.e. surface water and groundwater). By our method the evolution of PFAA concentrations has been followed during all treatments steps, including the presence of potential precursors which can generate PFAA after end-of-pipe oxidative treatment before the distribution as potable water. The results showed that the soil infiltration is able to generate PFAA from precursors already present in influent waters. The method for the estimation of PFAA precursors has been also applied to the discharges of two factories that produce or used fluorochemicals, which represent the most significant sources of PFAS for the Italian river basins. The method demonstrated to be very effective also in assessing the actual risk for the ecosystem cause by discharges of fluorochemical plants. This risk has been always underestimated because industrial processes produces a significant amount of precursors that can be *in vivo* transformed in PFAAs contributing to their environmental occurrence and bioaccumulation in the organisms.

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Mass flows of per- and polyfluoroalkyl substances (PFASs) in a wastewater network and treatment plant

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Per- and polyfluoroalkyl substances (PFASs) are man-made substances that hold unique properties. The effluents of wastewater treatment plants (WWTPs) have been pointed out as one of the major sources of PFASs in the environment. The main aim of this project was to evaluate the sources and the occurrence of PFASs in a wastewater network and in the different treatment steps at the connected WWTP. Another objective was to calculate mass flows and to investigate the fate of PFASs within the WWTP. Both wastewater and sludge were sampled including pumping stations (PSTs, n = 15) and at the WWTP (n = 10). The PFAS concentrations and composition profiles varied greatly in the network. High concentrations of 6:2 fluorotelomer sulfonate (6:2 FTSA) were found in the wastewater, which indicates usage of 6:2 FTSA in industrial processes and/or leaching from fire-training sites. A hot spot was detected (ΣPFAS = 55,000 ng L^{-1} = 110,000 mg d^{-1}) with elevated discharges of C_3–C_8 perfluoroalkyl carboxylates (PFCAs). The studied WWTP was found to be ineffective in removing C_4, C_6, C_8 perfluoroalkyl sulfonates (PFSAs), C_3–C_8 PFCAs and 6:2 FTSA from wastewater. Longer chained PFCAs (C_9–C_{17}) tended to partition to sludge more effectively than shorter chained PFCAs (C_3–C_8). PFSAs and PFCAs tended to be at similar concentrations in the effluent compared to the influent indicating insufficient removal of PFASs within the WWTP.

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Transport and Profiles of PFASs in the Arctic Ocean

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Water samples were collected and analyzed from different depths of the water column at several locations in the central Arctic Ocean, along the Arctic shelf and several snow and meltpond water samples. The aims of this study were to (i) understand the spatial and vertical profiles of PFASs in the Arctic; (ii) assess the role of atmospheric versus oceanic transport of PFASs into the Arctic Ocean and (iii) derive a mass budget of PFASs in the Arctic Ocean. A total of 69 samples were analyzed for 39 PFASs. Thirteen PFASs (C6-C12 PFCAs; C6, 8, 10 PFSAs; MeFOSAA and EtFOSAA, and FOSA) showed detectable concentrations (< 5 – 343 pg/L) in the samples. In general, detection of PFASs was limited to 150m below surface, except for the location North Barents Sea (PS80/227) that PFOS was detected up to the depth of 250 m below surface. Different locations exhibited different composition profiles. Different composition profiles of PFASs in water at the four sampling points may be explained partially by sources and circulation of the Arctic. No consistent composition profiles were observed from surface along the depth of water column. Similar composition profiles were observed in some layers of water columns. In the summer, with the sea ice melt and river run-off, the surface water of the Arctic forms the polar mixed layer (PML), which has a lower salinity compared to the deeper water layer (first halocline, then Atlantic layer). In present investigation, the depths of PML ranged from 10 to 30 m from surface, where we detected C6-C9 and C11 PFCAs, C4, C6, and C8 PFSAs. In the Atlantic water layer, only PFOS and PFNA were detected at location North Barents Sea (PS80/227), in which PFOS was detected at 11- 37 pg/L and PFNA at 13 pg/L; surprisingly, no PFOA was detected. This is the only indication we have so far that oceanic currents move PFASs into the Arctic Ocean. At the locations further from the Atlantic Ocean, such as Nansen Basin - West Gakkel Ridge (PS80/254), Amundsen Basin - East Gakkel Ridge (PS80/275), and near the North Pole (PS80/364), no detectable PFASs were observed in the Atlantic layer. The observed vertical profiles of PFAAs in the current investigation may be explained partly by the circulation and water sources of the Arctic. There is little evidence that ocean currents transported PFASs into the deeper Arctic Ocean layers, which remain mostly PFAS-free.\n

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Perfluoroalkyl substances in polar bears from Svalbard: Current knowledge on levels, trends and effects

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Perfluoroalkyl substances (PFAS) are among the major environmental contaminants in the Arctic ecosystems. Our study concludes the knowledge of levels, temporal trends and effects of PFAS in polar bears from Svalbard. We measured plasma concentrations of 12 PFASs in 199 adult female polar bears sampled throughout Svalbard in April each year during the period 2000-2014. We also analyzed carbon and nitrogen stable isotope ratios (δ^{15}N and δ^{13}C) in red blood cells and determined habitat quality for individual bears. In part of the bears, we analysed plasma clinical chemical parameters and transcript levels of genes in fat related to lipid metabolism. Furthermore, we built an in vitro assay to study activation of polar bear peroxisome proliferator receptor alpha (PPARA) by individual PFASs. PPARA is the major regulator of lipid metabolism in liver tissue. PFAS was quantitatively the major compound group in polar bear plasma. PFAS concentrations increased with increasing intake of marine vs. terrestrial carbon, and with trophic level and were higher in bears using areas with higher sea ice extent. The bears that were fasting had higher concentrations of all PFAS. Concentrations of PFHxS and PFOS in polar bears decreased between 2003-04 and 2009-10, after which they have been stable. PFNA, PFDA and PFUnDA increased from 2000 to 2014 ~3% per year. PFAS concentrations were significantly related to parameters related to lipid metabolism including transcript levels of genes in fat tissue and clinical chemical parameters in plasma (will be presented in detail by Sabrina Tartu). In addition, the major perfluoroalkyl carboxylate (PFCA) in polar bears, namely PFNA, activated polar bear PPARA in vitro at environmentally relevant concentrations. We conclude that PFAS are emerging contaminants in polar bears from Svalbard. PFCAs are increasing and PFOS concentrations have not declined the last years. Results from correlative field studies and in vitro studies indicate that these componds disturb polar bear lipid metabolism.

Wastewater effluents: How research can improve risk assessment and regulation

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CONCAWE - A 53 year history developing knowledge and research to improve water management and environmental risk assessment of petroleum oil refinery effluents.

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The conservation of Europe’s water resources was one of the main drivers, when CONCAWE (which stands for **CON**servation of **C**lean **A**ir and **W**ater in **E**urope) was constituted in 1963, following the commitment made by the Industry at the 6th World Petroleum Congress, in Frankfurt. At this time pollution of both air and water was becoming an international issue following the growing awareness that man was having a significant adverse impact on the environment which could no longer be sustained. Water remains an essential resource that has, over the years, come progressively higher on the international agenda because of its intimate relationship with both human health and ecosystem development. Petroleum oil refineries use large quantities of water and effective management of water, from supply through handling and treating to final discharge into the environment, remain a key requirement for the efficient and responsible operation of a modern refinery. In the 53 years of CONCAWE’s existence the refining sector can demonstrate that it has made significant improvements to its handling and treatment of water. The initial focus was to reduce oil discharges from refineries through the installation of increasingly sophisticated treatment systems. By undertaking regular surveys using uniform and reliable techniques CONCAWE can demonstrate that the achievements over the past 50 years are considerable with significant reductions in the discharge of oil and most other refinery pollutants. Since CONCAWE’s inception the challenges relating to water use have changed and new metrics are increasingly being used to assess oil refinery effluents. Over this period CONCAWE has collated data and managed research programmes to assess the potential impact of effluents using both chemical and biological measures to meet these new challenges. These programmes continue employing ever increasingly sophisticated methods to keep pace with changes in both scientific knowledge and societies expectations. Using a combination of robust survey data and scientific research has enabled CONCAWE to contribute to the development of improved techniques and methods to underpin the environmental risk assessment of discharges. These data also help to provide weight of evidence that the potential for adverse environmental impacts of discharges from the oil refining sector on Europe’s surface water bodies have significantly reduced commensurate with the reduction in pollutant loads.

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Understanding the fate of active pharmaceutical compounds in surface waters receiving poorly or untreated sewage effluent and the development of appropriate environmental risk assessment approaches

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Active pharmaceutical ingredients (APIs) have been classified as emerging contaminants, and their introduction to the environment might pose risks to ecosystems. The major source of APIs to the environment is human use and excretion or the improper disposal of unused medication to the sewerage system. In wastewater treatment works the inefficient removal of some pharmaceuticals, combined with their pseudo-persistence, results in their presence in surface waters. As patient access to medicines increases in lower income countries the environmental concentration of APIs has the potential to be higher than in high income countries if the level of wastewater and drinking water treatment does not also increase. Untreated wastewater enters the environment via discharge into surface waters resulting in a downstream area characterized by high pollution, and named the “impact zone”. Characterization of the environmental risk posed by APIs in such an area is challenging since the formal protocol for environmental risk assessment (ERA) was developed for environmental conditions largely different from the ones encountered in the impact zone; high levels of BOD, ammonia, and other potential toxic substances exist in combination with low concentrations of dissolved oxygen, meaning that there is an absence of traditional species used for toxicological endpoints. Therefore, the calculation of predicted environmental concentrations (PEC) in such conditions is unlikely to be relevant. The aim of this research is to obtain a comprehensive overview of the gaps regarding the fate of pharmaceuticals in aquatic environment initially starting with an extensive literature review of available data for (1) occurrence, (2) degradation rates, (3) partitioning to dissolved organic matter, colloids, suspended solid matter, and sediments, and (4) relevant endpoints. This will inform experimental research aimed at gaining data on partitioning and degradation of selected pharmaceuticals in the impact zone under varying dilutions and conditions, in order to derive more accurate endpoints. The information obtained will be used to develop an ERA approach for impact zones, as only with more accurate exposure concentrations and impact data can the risks to the aquatic environment of APIs be quantified.

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Advanced wastewater treatment for removal of micro-pollutants - a cure for the Baltic Sea?

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Many chemicals used in everyday life are collected in sewage that passes through wastewater treatment plants (WWTPs). Advanced wastewater treatment has therefore gained much attention as part of the strategy to protect the aquatic environment from so called micropollutants (MPs). A range of technologies exist that remove MPs from wastewater via physical separation, biological degradation and adsorption. Transformation of micropollutants aided by ozone (O3) or adsorption onto activated carbon (PAC or GAC) are the two technologies mainly tested/implemented in full scale. In this study the possibilities to evaluate the benefit of advanced wastewater treatment as a measure to reduce pollution of the Baltic Sea are explored. This in terms of possible reductions of **1)** *total MP load* and **2)** *concentrations* of MPs in WWTP effluents, with calculations based on available data on chemical concentrations in water and measured removal efficiencies in full-scale applications of advanced treatment technologies. Data on measured removal efficiencies (REs) of MPs reported in scientific publications were compiled. In addition, concentrations in influents and effluents of the MPs were compiled, both from scientific literature and the Swedish screening database. The average RE for MPs detected in effluents of in conventional WWTPs was ca 50-60%. The weighted average RE based on typical influent concentrations was higher, ca 95%. Advanced treatments (AC or O3) removed or transformed on average 60-80% of the MP mass, depending on dataset and if averages were weighted to typical effluent concentrations or not. It was estimated that an upgrade of all WWTPs located < 20 km from the Baltic Sea coast serving >100 000 population eqvivalents would likely reduce or transform around 40-60% of the total chemical load fom the coastal treatment plants. Average concentrations in Swedish effluents of compounds prioritized under the WFD were in most cases below the environmental quality standards set for marine waters. More data on REs in full scale applications for a wider range of MPs are, however, needed to enable better estimations of the total benefit of advanced treatment of wastewater.

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Occurrence, characterisation and fate of (nano)particulate Ti and Ag in two Norwegian wastewater treatment plants

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Due to their widespread application in consumer products, titanium (Ti) and silver (Ag) nanomaterials are increasingly released to urban wastewater treatment plants (WWTPs) from households and industrial facilities. In the current study, we assessed the occurrence of Ti and Ag in WWTP influents and their elimination in full-scale WWTPs employing preliminary and primary treatment steps. Influent Ti and Ag were characterised as (nano)particulate, colloidal and dissolved fractions using a size-based fractionation method employing sequential filtration. A seven-day sampling campaign was conducted in two full-scale WWTPs in Trondheim, Norway. Eight-hour composite influent samples were collected to assess diurnal variations in Ti and Ag occurrence, covering morning, afternoon/evening and night discharges. Daily influent and effluent samples were used to determine removal during wastewater treatment. Measured influent Ti concentrations (up to 211 $\mu g\ L^{-1}$) were significantly higher than Ag (< 0.15–2.2 $\mu g\ L^{-1}$). Most of the influent Ti and Ag occurred in particulate form or was associated with suspended solids (>0.7 μm), while 5% Ag and < 1% Ti was found to occur in colloidal or ionic form. Different diurnal concentration profiles were observed for the two elements. Ti profiles were comparable to flow and pollutant patterns in municipal wastewater (morning and/or evening peaks, night minima), indicating predominant emissions from households. Irregular profiles were exhibited by Ag, possibly determined by short-term discharges from one or more point sources (e.g. industry). Removal efficiencies of ≥ 70% were observed for both elements with the exception of one WWTP, where Ti removal was shown to be on average < 40%. These differences may be attributed to the different removal of suspended solids (where most Ti and Ag were found to occur) in the two WWTPs. The present findings will be complemented with identification and characterisation of Ti and Ag nanomaterials using transmission electron microscopy and X-ray photoelectron spectroscopy, and prediction of occurrence dynamics by means of influent generation algorithms. Overall, this study permitted (i) identification and tentative quantification of (nano)metallic Ti and Ag discharge from urban/industrial catchments, and (ii) determination of emissions from WWTPs to environmental compartments.

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Periphyton tolerance to micropollutant mixtures before and after upgrading of a wastewater treatment plant
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In Switzerland, wastewater treatment plants (WWTPs) are currently upgraded to reduce input of micropollutants (MPs) into the receiving streams by applying an additional treatment step such as activated carbon. This, offers the opportunity to study in real environments consequences of a reduction in MP concentrations. Therefore, the goal of this study was to examine tolerance to MP mixtures, extracted from passive samplers from WWTPs effluents, ofperiphyton that has been sampled one year before and after the upgrading of a WWTP, in comparison to impacts on periphyton from two non upgraded WWTPs. Periphyton was sampled up- and downstream of three WWTPs in 2014 (before upgrading) and 2016 (after upgrading) and exposed to increasing dilutions of extracts from passive samplers that were deployed at the effluent of each WWTP. Bacterial and algal tolerance to the extracts was determined by measuring the inhibition of ^{14}C-carbonate and ^{14}C-leucine incorporation, respectively. The diversity of up- and downstream periphyton was examined by performing a DNA fingerprint analysis. Moreover, 56 MPs were quantified in grab water samples from each up- and downstream location and in the passive samplers extracts. Results showed that one year after the upgrading, the difference in MP concentrations between up- and downstream highly decreased. These results concurred with the tolerance measurements at this site, since no difference between up- and downstream periphyton was observed, unlike in 2014 where higher algal tolerance was measured downstream than upstream. Moreover, similarly to the results from 2014, increased tolerance to the extracts was still detected in 2016 from up- to downstream of the non-upgraded sites. These results point to the strong relationship between microbial tolerance and the intensity of contamination by MPs. Microbial diversity was consistent with tolerance measurements. This was clearly the case at the non-upgraded sites in 2014 and 2016, where bacterial and algal diversity of up- and downstream periphyton were different. On the contrary, differences at the upgraded site were less pronounced in 2016, which was not the case in 2014, indicating that MPs were a major driver for the microbial diversity downstream of the WWTPs. Overall, our study provides a solid proof for the suitability of microbial tolerance measurements, coupled with passive sampling, to pinpoint specific effects of MPs from WWTPs at the community level in fresh waters.

Life Cycle Data and Modeling Developments: challenges and solutions

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Quantitative analysis of system models in an inventory database
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Uncertainty due to choices is one of the major components of uncertainty. In life cycle assessment (LCA), uncertainty due to choices appears mainly in the discretization of a complex and dynamic world to a set of activities resolved in space and time, and in the application of system models to these activities to produce a consistent, linked inventory database. Until recently, an systematic examination of these choices was quite difficult. Most system model software was proprietary, and raw data and metadata were not available. However, comparative attributional and consequential LCA studies have shown that these choices are significant. In order to test the effect of various system modelling choices, we have built a system modelling software framework for LCA called Ocelot. This software is free and open source, and well tested and documented. Ocelot is designed to allow for the construction and analysis of system models, and as such breaks modelling choices into a series of software functions. The ecoinvent "cut off" system model, for example, is more than 50 such functions. As each system model is built on a series of functions, each (mostly) independent of the other functions, the effect of each function can be tested independently. We turn off or modify each function in the “cut off” system model, and test the relative changes in LCIA scores between the base case and the system model variant for each sample dataset. In selected cases, we also assess the change in LCIA score uncertainty. In this way, we can provide quantitative estimates of the effect of each component of a given system model paradigm. Most debates over system models refer to broad, consistent paradigms for building worldwide models of human and natural activity. Our project allows us to complement this approach by also thinking of system models as a set of small and understandable software functions, each presenting a solution to a particular problem. We can now focus debate and research on how best to solve specific questions in each of the three main system model tasks.

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Representativeness of environmental impact assessment methods regarding life cycle inventories of products
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When analysing the environmental impact of a technical system with Life Cycle Assessment (LCA), practitioners face the difficult choice of the Life Cycle Impact Assessment (LCIA) method to use. We propose a Representativeness Index (RI) allowing an assessment of LCI information captured by the LCIA methods. It allows an objective appraisal of LCIA methods by additional information on the adequacy and the completeness representation of LCI they actually perform. A LCI describes any technical system throughout all its value chain by quantifying all emission flows to the environment and all consumptions flows of resources, both named elementary flows. From LCI to LCIA results, LCA methodology is a dimensional reduction technic where the resulting dimensions have an environmental meaning. LCIA reduces the complexity of systems described at LCI level by hundreds of variables (high-dimensional dataset of elementary flows, difficult to fully apprehend comparisons), to a reduced number of criteria where systems are described by their performance on a few environmental impact categories (low-dimensional dataset, easier comparison). However, the adequacy of the different LCIA methods can be questioned regarding the information of the LCIs. The EcoInvent 3.1 database and thirteen LCIA methods are used in the present work. Both of them belong to a vector space of 1,869 dimensions where each dimension represents an elementary flow. LCIs and LCIA methods can then be localised in this vector space. After data pre-processing, RIs are calculated with the absolute value of the cosines between the directions of LCIs and LCIA method sub-space. The higher a cosine is, the more correctly the LCIA method represents the contribution of environmental flows on the direction of the LCI. Four inventories referred to the production of 1 kWh of high voltage electricity mix are used to illustrate this methodology (countries and areas analysed: China, France, Germany and North-eastern North America). The RIs of the LCIA methods are calculated for those four LCIs. Results show that comparing France and NPCC electricity mixes is more precise by using Ecological Scarcity or the ILCD method but comparing Germany and China electricity mix is more accurate with ReCiPe (high RIs with low difference). Additionally to the classic criteria usually used to select LCIA methods, this analysis gives extra information by characterising LCIA methods in terms of representation of the LCIs.

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Introducing the LCA data machine
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In any LCA study, finding data sets that are “fit for purpose” is probably one of the aspects which consumes most time and effort, and the data sets which are used have obviously a direct implication on the stability of the result achieved. This high effort for finding and creating data sets is true despite the existence of several comprehensive, generic databases for LCA. It is underlined by the presence of intransparent, outdated, or out-of region and context data set in these databases. Altogether, this process is slow and error prone. Further, industry information is, in this approach, often seen as best information source “as such”, although recent revelations, related to diesel engine emissions for example, show that companies might be tempted to provide too optimistic and even inaccurate information. On the other side, more and more data are becoming available, from public sources for example directly; from other sources, information can be harvested, and data analysis tools help to detect patterns in data and to infer elements in a data set. Information theory concepts are available to help understand under which situations additional information improves the data set, and not the least recent developments in the UNEP GLAD initiative help to better understand data quality and fitness for purpose in LCA databases and data sets. All these aspects together led to the development of an “LCA data machine” at GreenDelta, which will be introduced in the presentation, first as a concept, and then in a real application. The LCA data machine automatizes creation, update, and to some extent also review of data sets in LCA and sustainability assessment. Data sets are created to meet several specific requirements, e.g. related to region, time, or nomenclature system, but can also be created so that they meet requirement sets, such as, for example, related to PEF. The LCA data machine has been developed at GreenDelta in the last 2 years; it will be explained and demonstrated in the presentation, by help of a data set for electricity generation from fossil fuel. The building of the dataset is shown, in several stages, as well as the resulting data set. The data set is compared to a data set for the same product, created following the ”classic” data creation approach. Finally, the

usefulness, application areas, and impact of the data machine on current LCA data practice and LCA application will be discussed.

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Global sensitivity analysis in LCA of emerging technologies: Accounting for inputs' variability
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Life Cycle Assessment (LCA) has been widely applied over the last three decades for the environmental assessment of products and processes. While LCAs are often based on available data from existing processes at pilot or large scale, about 80% of environmental impacts are linked to decisions at the design phase. The application of LCA framework to emerging technologies is subject to a high level of uncertainty. Global sensitivity analysis (GSA) is an available tool to address this uncertainty but its accuracy depends strongly on the description of the inputs' variability. In this work, we propose a protocol to evaluate the effect of the selection of inputs' descriptions on GSA results. The method starts from the application of previous GSA protocols to LCA models to obtain the ranking of key parameters, according to available methods based on the decomposition of the variance (i.e. Sobol' indices). We propose an intermediate step to analyze the influence of the description of each input. This intermediate step consists of the reiteration of GSA under alternative hypothesis regarding the description of the inputs' variability. The GSA results allow identifying the inputs for which the description influences the parameter ranking. When the descriptions identified as influent also present a low level of confidence, their refinement is highly recommended. To illustrate the application of the method, it was applied to a model for the estimation of greenhouse gas emissions from enhanced geothermal systems (EGS), an emerging renewable energy technology. The "baseline" GSA results provided an initial parameter ranking and the identification of the installed capacity of the organic Rankine cycle, the borehole depth and the number of wells as the 3 main parameters responsible for most of the variability of the GHG results. The results from 3500 GSA calculations (corresponding to the baseline and 34 additional combinations, with 100 bootstraps per set of distributions) showed that the descriptions of the borehole depth and the lifetime may cause variations on this ranking. As a result, 5 inputs were identified as key parameters and included in a simplified LCA model to obtain the GHG emissions of EGS from a parameterized equation. This approach will help avoiding inaccurate interpretations and increase robustness of GSA results. It is particularly suitable for emerging technologies and processes at early stages of process design.

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A second-generation life cycle inventory model for chemicals discharged in wastewater
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We present a new model and tool to calculate life cycle inventories (LCIs) of chemicals discharged in urban wastewater, WW LCI v2. It integrates the approach of SewageLCI, i.e. fate modelling of chemicals released to the sewer, with that of WW LCI v1, i.e. a complete life cycle inventory, including infrastructure requirements, energy consumption and auxiliary materials used for the treatment of wastewater and disposal of sludge, as well as emissions resulting from direct discharges. In addition, WW LCI v2 has been expanded to account for different wastewater treatment levels, i.e. primary treatment, secondary (biological) treatment, tertiary treatment (sand filtration) and independent treatment by means of septic tanks. As for sludge disposal, composting of sludge has been added as a management option in WW LCI v2, adding to the existing options of reuse in agriculture, landfilling and incineration in WW LCI v1. In order to reflect current wastewater treatment scenarios, the model includes a database with statistics on wastewater treatment levels and sludge disposal practices in 56 countries. The model is programmed in an Excel spreadsheet, which accommodates simultaneous calculations for 30 chemicals, either individually or as a mixture. The output of WW LCI v2 is a comprehensive inventory linked to ecoinvent v3 data sets, that can be imported to LCA software in order to complement a life cycle assessment study of a particular chemical or a chemical mixture associated to a product or service. Impact assessment calculations are then easy to perform for different impact categories. WW LCI 2.0 makes it possible to obtain a chemical-specific and comprehensive LCI of chemicals discharged down the drain, including not only treatment in the wastewater treatment plant (WWTP) but also sludge disposal and degradation of chemicals in the environment when there is no connection to a WWTP. This is particularly important since emissions from direct discharges can be important. The model provides substantially different outcomes depending on the expected behaviour and composition of chemicals as well as on the existing level of treatment where they happen to be discharged. This model takes a step forward from its predecessors, WW LCI v1 and SewageLCI, enabling a more realistic assessment of the impact of chemicals at their end of life, thus contributing to better decision making and data availability in the context of the life cycle of chemicals.

Higher tier approaches in the risk assessment of plant protection products and their links to protection goals

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A new pulsed-exposure early life stage test design for rainbow trout on an insecticide. Refining OECD Guideline 210 to meet the needs of EFSA Aquatic Guidance 2013
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Constant-exposure in OECD TG 210 Fish Early Life Stage studies is unrealistic for fast-dissipating pesticides compared to edge-of-field water-bodies. EFSA Aquatic Guidance (2013) allows aquatic toxicity studies to be modified so the exposure-profile (peak-height, pulse-duration, number of pulses) is comparable to the worst-case predicted field-exposure. In the present study on a synthetic pyrethroid (SP) insecticide, a novel method was developed to *simultaneously* assess effects on 3 early-life stages of rainbow trout. The TG 210 design was modified to incorporate a worst-case time-variable exposure profile in tanks containing a 10 mm sediment layer and stainless steel mesh barrier to allow water movement but prevent fish disturbing the sediment. Life stages used were newly fertilised 'eggs', 'alevins' (non-feeding larvae) and free-feeding 'swim-up' fry. To ensure physical separation of the 3 life stages within the tank, eggs and alevins were each held in a glass incubation tube with a mesh base. A control group plus 5 concentrations were used. To start, each group had 4 replicate tanks each with 50 eggs, 20 alevins and 20 swim-up fry. There were two 72 hour static exposure phases on Days 0 and 14. The study duration for organisms starting as 'eggs', 'alevins' and 'swim-up' fry was 72, 45 and 31 days respectively. This allowed for the assessment of effects over a period including at least 2 weeks of growth after initiation of free-feeding for each of the 3 life stages. Standard end points were assessed including hatch success, survival, growth and clinical signs (e.g. loss of equilibrium and coordination). To assess the potential neurotoxic action feeding behaviour was categorised as *active, passive* and *not feeding*. This refined-exposure study showed that 3 critical life stages of fish can be tested *simultaneously*, whilst complying with the fundamental elements of TG 210. Control hatch and survival rates were both > 95%. The design allowed the direct comparison of the sensitivity of each life stage to identical pulsed exposure profiles. Free-feeding swim-up fry was the most sensitive exposed life stage, based on clinical signs, feeding and slightly reduced growth. Swim-up of exposed alevins was delayed at high treatment levels. Exposed eggs were unaffected.

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Relative importance of waterborne vs. dietary exposure towards the neonicotinoid thiacloprid for leaf-shredders
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Neonicotinoids represent one of the most widely used insecticide classes worldwide. Their tremendous success is, amongst others, attributed to their systemic properties facilitating their rapid uptake and distribution within treated plants. Due to their high water solubility and high environmental persistence (in soils and plants), neonicotinoids may enter streams not only via surface runoff and spray drift but also within neonicotinoid-contaminated plant parts (e.g., leaves). In streams, particularly leaf-feeding detritivorous invertebrates may be affected through waterborne exposure and the consumption of contaminated leaves. To unravel the relative importance of these pathways, the key leaf-shredding invertebrate *Gammarus fossarum* (Amphipoda) was subjected to these paths employing a two-factorial design for 21 days. Its leaf consumption and lipid content were monitored as ecotoxicological response variables. Black alder leaves (*Alnus glutinosa*) containing environmentally relevant amounts of the neonicotinoid thiacloprid (THI; ~250 μg/g) were used to expose *Gammarus* either a) towards THI leaching from contaminated leaves into the water (= waterborne path), b) via the consumption of THI-contaminated leaves (= dietary path) or c) a combination of both (= combined path). Both the waterborne and diet-related exposure pathways significantly reduced gammarids' leaf consumption (by ~30 and ~35%) and lipid content (by ~22 and ~20%; only significant for the latter), when considered individually compared to a neonicotinoid-free control. The effects were more pronounced when subjected to a combination of both exposure pathways (i.e., 52 and 26%). Based on these results, adverse implications on the gammarids' physiological fitness and their role in the leaf decomposition process might be expected through the consumption of neonicotinoid-contaminated leaves even if concentrations in the surrounding medium are negligible. Moreover, as *Gammarus*

showed – independent of the exposure pathway – a capacity to accumulate up to ~400 ng THI/g, the diet-related pathway might also affect higher trophic levels. Consequently, neglecting dietary exposure might underestimate the environmental risk systemic insecticides pose to ecosystem integrity.

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Effects of imidacloprid on freshwater outdoor microcosms in Bangladesh

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Imidacloprid is a neonicotinoid synthetic insecticide and veterinary substance, widely used in Bangladesh for a variety of crop protection. Imidacloprid may enter into the adjacent aquatic ecosystems via spray drift, surface runoff, and ground water leaching and contaminate these ecosystems. The main objective of the present study was to assess the fate and effects of imidacloprid on the structural (phytoplankton, zooplankton, macroinvertebrates and periphyton) and functional (organic matter decomposition) endpoints of freshwater ecosystems in Bangladesh. Imidacloprid was applied in outdoor microcosms (PVC tank containing 400L water) at nominal concentrations of 0, 0.03, 0.3, 3 μg/L over a period of 4 weeks with four replicates per treatment. No observed effect concentrations (NOECs) for water quality variables, phytoplankton, zooplankton, macroinvertebrates, periphyton community and organic matter decomposition were determined using Williams test. Furthermore, phytoplankton, zooplankton and macroinvertebrate community were analysed using principal response curve (PRC) method. Univariate analysis showed significant effects of imidacloprid on water quality variables, some phytoplankton, zooplankton and macroinvertebrate taxa in the highest and second highest treatment level on different sampling days. No significant effects were observed on periphyton and organic matter decomposition (NOEC $\geq$ 3 µg/L). The principal response curve (PRC) did not show significant effects of imidacloprid on phytoplankton community ($p = 0.718$), but it did on zooplankton ($p = 0.023$) and macroinvertebrate ($p = 0.002$) community, thus indicating higher sensitivity of micro and macro invertebrates than of primary producers. The results of the study suggests that 0.03 μg/L of imidacloprid may have deleterious effects on some structural endpoints of freshwater ecosystems. This study generates safe environmental values of imidacloprid for the individual taxa and community levels of some endpoints through the derivation of NOECs. Our study determined some NOECs (< 0.03 ug/L) for certain taxa indicates that the standard of imidacloprid (0.03 ug/L) used in Europe might not protect freshwater communities in Bangladesh. We recommend further studies to get insight into the comparative toxicity of imidacloprid using the data obtained from this study with those have been previously available in temperate regions.

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Proposal of a higher tier non-target plant study design

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In agricultural areas, natural occurring plants and herbs growing at field margins can be affected by agrochemicals used at the nearby fields. In the EFSA Opinion for non-target terrestrial plants (2014) it was proposed, that one important goal of protecting non-target plants is the protection and maintenance of the biodiversity which also supports the abundance and biodiversity of many non-target arthropods or birds. As stated in this opinion, the actual procedure of testing the effects of plant protection products on non-target plants, has some open questions. Most testing is done in Lab or greenhouse with individually or in monoculture growing annual crops. The life-cycle with flowering, seed production or viability is not included within the tests. Few field studies have been done and no standard protocol exists for higher tier studies. Here a possible design to investigate the effects of pesticides to specific plant species and plant communities is presented. The study design with non-target plants tests drift rates under field conditions and assess individual species as well as the plant community. The study site was located in southwest Germany. Before the start it was sown with a commercial available seed mixture for flowering strips, containing 30 species. The area was separated into individual plots. The design consists of one control and 4 different herbicide rates, with 8 replicates, each. The highest rate tested was the field rate. Assessed were species abundances, plant cover and biomass, additionally phytotoxicological effects were recorded. For selected species the number of flowers and plant height were recorded in order to assess differences in development and flowering. Also, seeds were sampled, to assess possible differences in seed quantity and quality. To assess effects to the community a principal response curves (PRC) for the plant community was calculated. It showed significant impacts on the community down to 10% of the field rate until the end of the study. Further data analysis is still in progress and the outcome will be presented. **EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2014. Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target terrestrial plants. EFSA Journal 2014;12(7):3800, 163 pp. doi:10.2903/j.efsa.2014.3800**

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Opportunities to advance flexible and effective spray drift mitigation: Continuity and progress from SETAC MAgPIE to SETAC DRAW

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Risk mitigation measures are a key component in defining the conditions of use of pesticides in crop protection according to Regulation (EC) 1107/2009. They arise via the product risk assessment and are defined on a per-use basis, to be reproduced on the labeling following harmonized conditions as in Regulation (EU) 547/2011. In Europe, the question raises multiple exchanges between regulatory authorities, and many initiatives have been undertaken in order to develop, implement and account for risk mitigation measures in the risk assessment procedures through dedicated high-tier risk assessments. The MAgPIE workshop was organized under the auspices of SETAC and the European Commission. The workshop gathered risk assessors and risk managers within 21 European countries, industry, academia and agronomical advisors/extension services, in order to provide European regulatory authorities with a toolbox of risk mitigation measures designed for the more flexible management and use of pesticides for agricultural purposes, and thus contribute to promoting policy and practice harmonization within Europe. One component of the workshop involved the assembly of risk mitigation measures for spray drift and characterisation of regulatory status for acceptance and implementation – both in high-tier risk assessments and adoption by users of plant protection products. With the completion of the efforts of SETAC MAgPIE a need was identified to continue these technical and regulatory discussions on drift representation, mitigation and management. In 2016 a follow-up DRAW workshop was established under the auspices of SETAC. One objective of the DRAW workshop is to build upon the foundation of MAgPIE and continue progress with the risk mitigation measure toolbox, including a range of efforts; Expansion of the toolbox Characterisation of measures Proposals for harmonization (testing and representation) Strategies for support for dedicated high-tier risk assessment Support for more flexible and effective risk management and labelling This contribution will present the MAgPIE drift mitigation toolbox and discuss challenges highlighted during workshop discussions and the recommendations for spray drift management. This presentation will then expand on this foundation to summarise strategy in SETAC DRAW for continued efforts to expand and improve this toolbox and its implementation in high-tier risk assessments and by product users.

Linking Oceans and Human Health: a new trans-disciplinary research challenge

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The ocean and human health: a European perspective on an emerging integrated meta-discipline

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There is a growing body of evidence that the health of the ocean and that of humans are inextricably linked and that how we interact with and affect our ocean and seas will significantly influence our future on earth[1]. This relationship is growing increasingly important in light of rapidly growing coastal populations and climate change. Ocean and human health interactions include both risks and benefits. Extreme weather events such as coastal storms and flooding, and human exposure to marine-borne pathogens and to chemical and material pollution, pose significant threats to human health. At the same time, the seas provide numerous benefits to human wellbeing in the form of ecosystem goods and services including food, water, energy, climate regulation, bioremediation of human pollutants, and a host of recreational and cultural benefits. Biotechnology is opening opportunities to exploit marine genetic resources with potential for new drugs and nutraceuticals. Research is also beginning to identify the health-promoting effects of interacting with the coastal environment. However, understanding these manifold and complex interactions can only be achieved through interdisciplinary research, drawing from expertise across a diverse range of disciplines within natural, social and economic sciences, including public health and medicine. In 2013 the European Marine Board (EMB) published a position paper entitled, *Linking Oceans and Human Health: A Strategic Research Priority for Europe*[2]. This presentation examines the emergence of oceans and human health as an integrated meta-discipline in the context of the broader European and international policy landscape. Lora Fleming, Michael Depledge, Niall McDonough, Mathew White, Sabine Pahl, Melanie Austen, Anders Goksoyr, Helena Solo-Gabriele, and John Stegeman (2015). The Oceans and Human Health. Environmental Science: Oxford Research Encyclopedias. DOI:10.1093/acrefore/9780199389414.013.1 European Marine Board (2013). Linking Oceans and Human Health: A Strategic Research Priority for Europe. Position Paper 19 of the European Marine Board, Ostend, Belgium

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Red tides, Aerosols and Human Health

L. Fleming, University of Exeter Medical School / European Centre for Environment and Human Health

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Microbial pollution, antibiotic resistant bacteria, and Surfers
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Faecal pollution regularly introduces microorganisms such as bacteria, including antibiotic resistant bacteria, to coastal waters. People come into contact with these potentially harmful microorganisms when they participate in recreational activities in the sea. In high-income countries, such as the UK, there is a perception that the risk to human health of sea bathing is negligible. However, antibiotic resistant bacteria are an emerging threat, and few studies have sought to link exposure to antibiotic resistant bacteria in natural environments to people's health. In the Beach Bum Survey, we conducted an exposure risk assessment to quantify water users' exposure to one type of antibiotic resistant bacteria: CTX-M-producing *Escherichia coli*. This was followed by a cross-sectional survey of 143 regular surfers and 130 non-surfers to measure the prevalence of faecal carriage of these particular resistant bacteria in the two group. Surfers were four times as likely as non-surfers to be faecal carriers of CTX-M-producing *E. coli* (relative risk=4.09, 95% confidence interval 1.02 to 16.4, p=0.046). While small, this is the first study to investigate the link between human exposure to antibiotic resistant bacteria in coastal waters and gut colonisation. This is the first step towards understanding whether transmission of antibiotic resistant bacteria occurs in coastal waters, which is critical for developing effective strategies to reduce the spread of resistant bacteria.

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Blue Gym/Blue Health!
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Marine biogenic chemicals, cell signaling and human health
M. Moore, Plymouth Marine Laboratory, Plymouth University and ECEHH University of Exeter medical School
Living and taking recreation in rural and coastal environments promote health and wellbeing, although the causal factors involved are unclear. It has been proposed that such environments provide a counter to the stresses of everyday living, leading to enhanced mental and physical health. Living in natural environments will result in airborne exposure to a wide range of biogenic chemicals through inhalation and ingestion of airborne microbiota and particles. The "biogenics" hypothesis formulated here is that regular exposure to low concentrations of mixtures of natural compounds and toxins in natural environments confers pleiotropic health benefits by inhibiting the activities of interconnected cell signalling systems, particularly PI3K/Akt/mTORC1. When overactive, Akt and mTOR (mTORC1) can lead to many pathological processes including cancers, diabetes, inflammation, immunosuppression, and neurodegenerative diseases. There is a substantial body of evidence that many natural products (i.e., from bacteria, algae, fungi and higher plants) inhibit the activities of these protein kinases. Other mTOR-related interconnected metabolic control "switches" (e.g., PTEN & NF-κB), autophagy and other cell-protective processes are also affected by natural products.

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Occurrence and effects of bio-active substances in marine aerosols
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Throughout history, coastal environments have been linked to the promotion of human health. Today, epidemiological studies continue to observe that coastal populations are healthier and have longer life-expectancies than urban populations. At the same time, marine (eco)systems are under pressure by global change, the introduction of invasive species, overfishing and eutrophication. The relative recent increase of harmful algal blooms (HABs) is believed to be a consequence of these anthropogenic disturbances. About 100 HAB-species are classified as toxin producing algae, able to cause human intoxications via several exposure pathways. In contrast to the **consumption of contaminated fish and shellfish,** the phycotoxin exposure via the **inhalation of sea spray aerosols** (SSAs) is nearly undocumented. Exposure to brevetoxins and ovatoxins via SSAs has demonstrated to cause severe respiratory irritation in vulnerable groups living around the Gulf of Mexico and the Mediterranean. However, such an exposure does not have to be strictly negative. Indeed, it was recently hypothesized that the improved health in coastal areas could be related to the regular exposure to low levels of airborne biogenic compounds (**biogenics hypothesis**). To test this biogenics hypothesis we conducted a series of proof of principle experiments. We examined the effects of this respiratory exposure by exposing human lung cell lines for two days to several phycotoxins. Using viability assays and the analysis of specific protein pathways, we obtained the first experimental results supporting the biogenics hypothesis.

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Frequent multi-toxin (HAB) exposure through seafood consumption: an under-estimated risk to human health?
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Various anthropogenic impacts on the marine environment have caused a global increase in harmful algal blooms. The associated toxins cause respiratory and gastrointestinal diseases. Consumption is the best known route of human exposure to marine toxins. For this reason, food safety organizations employ limits of safe consumption for seafood, that were designed to protect against acute toxicity effects. These, however, do not account for chronic repeated exposure, nor for mixed toxicity effects. Moreover, there are still toxins for which no legal limit has been established. Combined, this creates a risk for consumers of fish and shellfish. For more than half a century, seafood monitoring programs employed the mouse bioassay as a quick, sensitive and specific method to detect potential hazards to humans and animals. For ethical reasons, this method is rapidly being replaced by analytical alternatives such as Liquid Chromatography tandem Mass Spectrometry (LCMS). While this alternative technique increases the sensitivity dramatically, it comes with a significant drawback: it fails to protect against unidentified toxins or unknown mixed toxicity effects. Here, we use UHPLC-MS/MS to show that toxin mixtures – at levels below legal limits – are common in North Sea biota. Using the blue mussel *Mytilus edulis* as a model organism, we illustrate that mixed exposure to several toxic species affects the metabolic processing of toxins, creating new metabolites which have unknown mixed toxicity effects on the health of human consumers.

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Discussion

Combined effects of chemical and environmental stressors: from local stressors towards climate change (II)

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Strong delayed interactive effects of metal exposure and warming: latitude-dependent synergisms persist across metamorphosis
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Predicting and understanding the impact of contaminants under warming remains a key challenge for risk assessment. Ignoring synergistic interactions between contamination and warming, delayed effects and differences in sensitivity across species' ranges could lead to an important underestimation of contaminant effects. We addressed all three mechanisms by studying effects of larval exposure to warming and zinc before, during and after metamorphosis in Ischnura elegans damselflies from replicated high- and low-latitude populations. By integrating delayed effects, synergistic interactions between contamination and warming, and differences in sensitivity across species' ranges in a single study we could identify two novel patterns: (i) post-metamorphic lethal effects of zinc were more important than the mild lethal effects during the larval stage and moderate effects during metamorphosis, and (ii) the well-known pattern that trace metals are more toxic at higher temperatures was latitude-specific, and particularly the dealyed synergism across metamorphosis was only present in low-latitude damselflies. This highlights that a more complete life cycle approach that includes the possibility of delayed interactions between contaminants and warming in a geographical context is crucial for a more realistic risk assessment in a warming world.

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The Interaction between environmental temperature and metal toxicity tolerance in zebrafish (Danio rerio)
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Zebrafish has become a valuable vertebrate model organism in a wide range of biological research including ecotoxicology. Although there are several studies on metal toxicity in zebrafish, investigations on the interaction between thermal background of the organism and metal uptake and toxicity are rare. Changes in environmental temperature can profoundly change species physiological condition. By this study we aim to see if different temperature regimes affect the organism tolerance against metal toxicity. For this purpose, we have defined two main scenarios**: 1-metal exposure subsequent a thermal challenge:** this scenario included acute and chronic thermal challenges in 5 different temperatures (17, 22, 25, 32 and 34 °C) and subsequently after the thermal challenge fish were introduced to metal contaminated water including Cu, Cd or Cu+ Cd for 10 days in 28°C**.**
2-metal exposure in a constant thermal challenge: After acclimatizing fish to 5 different temperatures for 4weeks they were exposed to metals for 10 days while the thermal challenge was lasting. The condition of the fish was monitored during

the experiment, including behavioral and physiological metrics and also water quality monitoring. At the end of the exposure period, whole body metal and essential electrolyte concentrations were determined using ICP-OES or ICP-MS. The results showed that Cu was much more toxic than Cd. However, Cu and Cd together showed a large synergistic effect. A low temperature shock appears to be protective while a high temperature shock increases sensitivity. Whereas, acclimation to warm temperatures increase the tolerance against metal toxicity in comparison to acclimation to cold temperatures. The results of whole body metal analysis showed that the metal burden in heat shocked fish is significantly higher than the cold shocked ones. While, the fish which acclimatized to cold temperature show a higher metal level in comparison to the fish which acclimatized to a warm temperature. Considering the major role of electrolytes in keeping the body homeostasis constant. We have also investigated electrolyte levels (Na, K, Ca and Mg). We found a consistent and significant drop in sodium levels between the alive and dead fish. Such an effect was not observed for the other major cations. The results of these experiments show that the thermal prehistory plays an important role in determining the tolerance of zebrafish towards metal exposure and likely also other stressors.

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Multi-generation toxicity of metal(loid) mining soils to Enchytraeus crypticus under the influence of climate change

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The aim of the present study was to assess the effects of climate change (increased air temperature and reduced soil moisture content) on the toxicity of a soil polluted by metal(loid) mining wastes to the soil dwelling organism *Enchytraeus crypticus* over three generations standardized on physiological time. A metal(loid)-polluted soil was collected from the mining district of La Unión-Sierra de Cartagena in SE Spain (pH ~6.2, total As ~640 mg kg^{-1}, total Cd ~21 mg kg^{-1}, total Co ~9 mg kg^{-1}, total Cu ~180 mg kg^{-1}, total Mn ~3000 mg kg^{-1}, total Ni ~16 mg kg^{-1}, total Pb ~6100 mg kg^{-1} and total Zn ~8100 mg kg^{-1}). Synchronized juvenile enchytraeids were exposed to a dilution series of the polluted study soil in Lufa 2.2 soil under different combinations of air temperature (20 °C and 25 °C) and soil moisture content (corresponding with 50% and 30% of the soil water holding capacity -WHC-) over three generations standardized on physiological time. The pH of Lufa 2.2 soil was adjusted with $CaCO_3$ to approximately 6 to avoid changes in metal availability when diluting the polluted study soil. Four climate scenarios were simulated: 1) 20 °C+50% WHC (standard conditions for bioassays), 2) 20 °C+30% WHC, 3) 25 °C+50% WHC, and 4) 25 °C+30% WHC). Adult survival and reproduction were checked as endpoints. Enchytraeid generation time was shorter with increasing air temperature and/or soil moisture content. Adult survival was only affected at 30% WHC, regardless of the air temperature (~30% reduction at the highest percentages of polluted study soil). Reproduction decreased with increasing percentage of polluted study soil in a dose-related manner and over generations (64-100% reduction at the highest percentages of polluted study soil). Soil toxicity increased at 30% WHC, regardless of the air temperature (50-74% reduction in EC50 in F0 and F1 generations), and over generations in the treatments at 20 °C (40-60% reduction in EC50 in F2 generation). At 25 °C soil toxicity did not change over generations when combined with 30% WHC and only slightly increased with 50% WHC. The present study shows that: 1) the generation time of *E. crypticus* changes when changing air temperature and/or soil moisture content, 2) the toxicity of a metal(loid)-polluted soil to *E. crypticus* not only depends on the pollutant level but also on the climate factors (air temperature and soil moisture content) to which the organisms are exposed, and 3) the toxicity changes over generations.

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Rapid thermal evolution shapes susceptibility to ZnO nanoparticles under global warming: a resurrection study of Daphnia magna

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Global warming not only challenges the ability of organisms to adjust to higher temperatures, but also their ability to deal with pollutants. Whether organisms will suffer from increased toxicity of pollutants under warming will depend on thermal evolution. Besides effects on life history, effects on body stoichiometry are important as these may link effects at the organismal level to ecosystem functions through nutrient cycling. We studied combined effects of warming and nano-ZnO in *Daphnia magna*, and aimed to test thermal evolution and its ability to offset the higher toxicity of ZnO at higher temperatures. *Daphnia* clones were resurrected from two periods from the same lake differing in temperature and were exposed to nano-ZnO at 20°C and 24°C. Results showed nano-ZnO only decreased the intrinsic population growth rate ('r') at 24°C, indicating a strong ZnO × Temperature synergism in the old population. In sharp contrast, this synergistic effect was not present in the recent population, which indicates thermal evolution not only improved the ability to deal with the higher temperature, but also offset the synergism between nano-ZnO and warming. Similarly, nano-ZnO only decreased the RNA:DNA ratio at 24°C in the old population, while this synergism was not observed in the recent population, indicating that also at the physiological level thermal evolution could offset the synergism between nano-ZnO and warming. Interestingly, nano-ZnO increased the %P and decreased the N:P and C:P ratios only at 24°C in the recent population. Changes in the body stoichiometry may have important consequences that may scale up to affect nutrient cycling in ecosystems. We provide the first experimental evidence that a higher toxicity to a pollutant (here a nanomaterial) at higher temperatures may be offset by thermal evolution. In other words, we demonstrated evolution of the synergistic interaction between a pollutant and warming, which has been ignored in the ecological risk assessment of pollutants in a warming world. Our results also highlight the potential of nanomaterials to modify body stoichiometry of *Daphnia* which may have important consequences for aquatic food web dynamics.

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Interactive effects between temperature and silver nanoparticles on microbial decomposers

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AgNPs can have toxic effects on aquatic species and compromise important ecosystem processes, even at environmentally realistic concentrations. AgNPs impacts have been the focus of much research, but their effects under different environmental scenarios, such as increasing water temperature are difficult to predict. The aim of this study was to evaluate the interactive effects of AgNPs and temperature on the activity and diversity of microbial decomposers of plant litter in streams. Litter-associated microbial communities were exposed in microcosms to increasing concentrations of AgNPs (50 to 75.000 μg L^{-1}) and $AgNO_3$ (5 to 7500 μg L^{-1}) and kept at 10ºC, 16ºC and at 23ºC. Leaf mass loss, fungal biomass and reproduction as well as microbial diversity were determined after 21 days. Also, the stress induced by AgNPs and $AgNO_3$ on litter-associated microbial communities was assessed by measuring the activity of fungal extracellular enzymes. Leaf mass loss was stimulated by the increase in temperature, while fungal biomass and reproduction were not. Increased AgNPs and $AgNO_3$ concentrations inhibited reproduction and diversity of fungi, particularly at the highest temperature. The activity of extracellular enzyme phenoloxidase was generally higher at 23ºC. The structure of microbial communities was more related to AgNP and $AgNO_3$ concentrations than to temperature. Changes in temperature not only change the physicochemical properties of AgNPs, but also exacerbate the AgNPs and $AgNO_3$ impacts on microbial decomposer activity. Our research highlight the importance of studying the AgNPs behavior under different environmental conditions, and the importance of studies at a community level to better understand the potential risks to freshwater biota and key ecosystem processes.

Wildlife ecotoxicology: cumulative effects through the food chain to the community

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Modelling species sensitivity distributions for POP-contaminated wild birds using quantile-regression

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In ecological risk assessment, SSD-models are commonly used in evaluating toxicity of compounds. These distributions are typically based on species- and compound-specific effect concentrations (e.g. EC_{50}s or NOECs). By cumulatively plotting effect concentrations for multiple species, individual species' effects are extrapolated to complete communities. Yet, the vast majority of SSDs is based on NOECs obtained from lab toxicity experiments including only aquatic organisms, as lab experiments on birds and mammals are limited. Therefore, documentation concerning effective toxicity in birds relies on observational data, which may have many drawbacks as many unmeasured potential factors might limit the birds measured response. To account for these factors, quantile regression analysis is a generally used approach, fitting data at a high quantile, assigning the majority of the response to limiting factors other than studied. Although this approach has been frequently used in ecology, it has rarely been used in the construction of ecotoxicological dose-response curves before. The main aim of present study was to construct field-based SSDs, based on EC_{10}s (as a NOEC-analog) derived from dose-response curves of wild bird species, using quantile regression. As especially persistent organic pollutants (POPs) have shown to have a big impact on temperate bird populations, due to reproductive impairment caused by eggshell thinning and hormonal disruption, this study was limited to these compounds only. Eventually, from these SSDs, HC_5s (hazardous concentration at which 5% of the species was affected) were obtained, that are suitable as thresholds in risk assessment.

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Neurotoxic effect of mercury on Arctic Barnacle goslings assessed in a controlled field study

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Although the Arctic region is remote from densely populated areas, it is contaminated by mercury from different sources. Via long range transport, mercury is transported to the Arctic via the atmosphere. Additionally, human activities may have contaminated the local environment. Mining activities for instance, have resulted in elevated levels of mercury in the soil. The focus of mercury-related research in the Arctic has mainly been on marine ecosystems, which are considered to be most at risk. Risks associated with mercury in terrestrial ecosystems have been less studied. Here we present a study, in which we raised human imprinted barnacle goslings (*Branta leucopsis*) in a contaminated mining site and a control site near Ny-Ålesund, Spitsbergen. Raising goslings by humans made it possible to perform a controlled field study, quantifying the accumulation of mercury in the goslings. Soil in the mining area contained significantly higher total mercury concentrations than the control site. This was also the case for the vegetation (approx. 2.2 times higher in the mining site than the control site). Each group of goslings grazed approximately 3-4 hours per day in their assigned area. At night and during bad weather conditions they were kept in pens. Goslings, which grazed in the mine impacted area showed increased levels of total mercury in the liver in comparison to the control group (ANOVA, n=8, paired for siblings, $p < 0.05$, applies to all statistical analyses). Although concentrations were relatively low, likely due to growth dilution in growing feathers, dopamine receptor levels in goslings were related significantly to hepatic mercury concentration. Furthermore, the behaviour of the goslings from the mine-exposed group was significantly different from the control goslings. The exposed goslings showed significantly more attempts to escape (jumps) when individually isolated for 20 min. This study indicates that, although exposure of organisms to mercury in terrestrial Arctic ecosystems in this case was relatively low, it still results in neurotoxic effects and even effects on the organisms' behaviour. In case of barnacle goslings this may have grave consequences in, e.g., interactions between parents and offspring. This warrants further studies, integrating mechanistic biochemical ecotoxicological observations with ecological relevant observations in behaviour and other fitness parameters.

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Using American dippers Cinclus mexicanus to investigate the influence of run-of-the-river dams on mercury exposure and mountain stream food webs.

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Run-of-river (RoR) dams are an increasingly common alternate energy source on mountain streams. Despite reductions in size and greenhouse gas emissions compared to conventional impoundments, RoR hydro may have ecotoxicological impacts through disruption of the natural flow regime. The American Dipper (*Cinclus mexicanus*) is a high trophic-level river bird that occupies mountain streams year-round and is a well-described indicator of stream health; thus, it is an ideal species to study impacts of RoR hydropower on river food webs. During 2014 and 2015, we conducted seasonal river bird surveys and sampled food webs at 7 regulated and 7 unregulated streams in coastal British Columbia, Canada. Regulated streams supported higher densities and consistent occupancy of dippers during breeding and non-breeding seasons, suggesting year-round residency occurs at high elevation streams stabilized by RoR-regulation. Analyses of dipper whole blood revealed significantly different isospace of birds between stream types ($p=0.032$), driven by significantly lower invertebrate $\delta^{34}S$ below RoR dams ($p=0.01$) and consequently ^{34}S-depleted blood at regulated streams. The bacteria responsible for ^{34}S-depleted food webs also methylate inorganic mercury into its toxic and bioavailable form, methylmercury (MeHg). Despite our observation of distinct dipper isospace between stream types, there was no model support for differences in mean dipper blood (417.55 ± 74.07ng/g ww at regulated streams, 340.73 ± 42.73ng/g ww at unregulated streams) or feather (1564 .55 ± 367.18 ng/g dw regulated, 1149.01 ± 152.10ng/g dw unregulated) mercury levels between stream types, with the exception of one recently regulated stream (Douglas Creek, Harrison Watershed). Douglas creek supported dippers with MeHg levels of potential toxicity concern (up to 8.5µg/g dw in feathers and 1.8µg/g ww in whole blood). Blood ($\beta=0.47$, $t=6.99$, $p< 0.001$) and feather ($\beta=0.40$, $SE=0.080$, $p< 0.001$) mercury levels in dippers at this stream were significantly higher than unregulated streams. With a negligible salmon contribution to the dipper diet and the absence of a known anthropogenic Hg point source, the elevated mercury levels recorded in American dippers at this regulated stream were best explained by demonstrated methylmercury production within the headpond and slight diet shifts.

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Functional role of diet as conditioning dilution or amplification effect on trace metal exposure in mammals: the case of a generalist rodent, Apodemus sylvaticus

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In terrestrial ecosystems, many chemical pollutants are mainly transferred within food webs. Even though the number of trophic levels is a key factor for determining the biomagnification of pollutants along the food chains, the importance of community diversity within a given trophic level on pollutant transfer to a higher trophic level is an open question. Moreover, the role of food in metal transfer could differ among resources according to their metal accumulation capacity. This study assesses firstly what is the relationship between oral exposure of small mammals to trace metals (TMs) and dietary richness, and secondly whether certain resources consumed can modulate such dependence between exposure and dietary richness. The study was carried out in an area impacted by a former smelter in northern France. Four forested areas of 25 ha were chosen along a soil contamination gradient. Wood mice *Apodemus sylvaticus* were sampled in spring and autumn 2012. Stomach content (SC) was extracted both for quantifying the concentraion of three TMs (cadmium (Cd), lead (Pb) and zinc (Zn)) as an assessment of the oral exposure, and for determining items consumed using the DNA based identification *metabarcoding*. Linear relationships between exposure to TMs and dietary richness were analysed according to soil contamination levels and to the presence/absence of main and preferred plant resources in SC of the animals. For exposure to Cd, the linear relationship between TM concentrations in SC and dietary richness was conditioned by the presence of *Salicaceae*, which are considered as hyperaccumulators. When Salicaceae were present in the diet, the exposure was negatively correlated with dietary richness of both plants and invertebrates. The relationship for Pb, a less mobile metal than the others, differed significantly according to soil contamination levels: the exposure was positively correlated with dietary richness of plant OTUs in the moderately-highly contaminated squares. The relationship for Zn, an essential trace metal, was conditioned by neither items nor soil contamination levels. Those results indicate that the exposure of wood mice to TMs is correlated with the number of items consumed, according to soil contamination levels and to the consumption of some resources such as *Salicaceae*. The observed effects might be controlled by the variations of TM accumulation capacity of resources within the communities of resources, which largely differ for each metal.

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Is rodenticide use disrupting the natural regulation of vole populations? A biomathematics modelling approach

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Anthropogenic activities modify ecosystems and as a result some ecological relationships are altered. For instance, many agricultural areas have followed a process of intensification. In these areas, voles frequently achieve pest levels through cyclic outbreaks reducing the food available for livestock and crop yields. Subsequently, some farmers respond using anticoagulant rodenticides to control voles. Thus, predators may be indirectly controlled by secondary exposure to rodenticides or by a reduction of food sources. Therefore, anticoagulant rodenticides might be acting analogously as a superpredator, interfering in the natural regulation of vole populations. Under what circumstances this may arise is not known. The objective of this study is to understand the interactions between an anticoagulant rodenticide (bromadiolone) to control vole populations and its indirect effects over vole specialist predators (stoats *Mustela erminea* and weasels *Mustela nivalis*)and a generalist predator of voles (the red fox *Vulpes vulpes*). We use a biomathematics modelling approach based on differential equations to explore several scenarios of this system. To study this we developed a model with two coupled dynamics: (i) the three studied animal populations, and (ii) their bromadiolone concentration. The four scenarios used are: *Scenario 1*, no bromadiolone use and no foxes; *Scenario 2*, bromadiolone use but no foxes; *Scenario 3*, no bromadiolone use but foxes; and *Scenario 4*, bromadiolone use and foxes. We observed the behaviour of the parts of the system until dynamics stabilisation. Our findings in *Scenario 1* show a population decline of voles up to an equilibrium in relation to a mustelid numerical response. In *Scenario 2*, we found a quick decline of voles and mustelids, followed by their recovery. In *Scenario 3*, there were stable vole dynamics and small effect of predation over voles. In *Scenario 4*, we found a decline and delayed recovery of all species. The highest final vole densities appear in *Scenario 3 and 4*, with red foxes, which are affected by bromadiolone use but also behave as generalist predators with wider food spectrum and thus less exposure. They also feed on small mustelids, specialist predators of voles. The next steps of this study will be to model additional scenarios, using new parameters and sensitivity analyses. The future modelling results will be compared with field results in areas where bromadiolone showed red fox population decreases.

Advances in the Environmental Fate of Down-the-Drain Chemicals, including Pharmaceuticals

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10 years experiences with fate assessment for human pharmaceuticals - new approaches available?

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In view to the revision of the 'Guideline on the environmental risk assessment of medicinal products for human use' (EMEA/CHMP/SWP/4447/00, June 2006, rev. 2) the current fate assessment is highly discussed concerning the exposure route via Sewage Treatment Plants (STP) for human pharmaceuticals which is mentioned as "poorly adressed" by some stakeholders. According to the current guideline, the exposure route via STP is considered by the test on ready biodegradability (OECD 301) in Tier A. This approach is in discussion since OECD 301 is only a screening and no simulation test for degradation in STP which gives qualitative default values for calculations of the behaviour in STP and no detailed information on primary degradation or transformation products during the processes in activated sludge. UBA evaluates the data received in the last 10 years for finding the gaps in fate assessment but also the benefit for the overall risk assessment. It will be considered if new approaches were feasible and if the current approach is yet suitable for human pharmaceuticals, The available data of the OECD 301 shows that only few human pharmaceuticals of the reviewed applications in the last 10 years are readily biodegradable. But from a regulatory perspective one great advantage of the OECD 301 is the possiblity of classification on biodegradation. Referring to the available data of OECD 308 tests around 70% of the tested active substances were classified as persistent in one of the environmental compartments. Most of them were persistent in sediment. The suggested OECD 314B is a best case test with low amount of the active substance and high amount of (adapted) activated sludge, performed at higher temperatures than ocurring in activated sludge of STP. Using such a less conservative test for prospective risk assessments is questionable for regulators. Referring to the exposure route it is to discuss if tests like OECD 314B could be an option for PEC refinement in Tier B or if it possible to derive also trigger values for such tests in Tier A. At present it seems that a classification is not foreseen for STP simulation tests which makes it difficult to include them in Tier A. Current monitoring data in the whole EU shows that many human pharmaceuticals are present in surface and ground water which requires also an assessment of the fate in the natural environment.

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Ten years' experience with the environmental risk assessment of human medicinal products

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The European Medicines Agency (EMA) guidance for the Environmental Risk Assessment (ERA) of human medicinal products has been in place now for over 10 years. The introduction of this guidance marked a step change in the ERA requirements for human medicinal products, with a shift from short-term acute to long-term chronic environmental effects assessments, and tailored ERAs for active pharmaceuticals ingredients (APIs) with suspected or known reproductive effects. In 2016 EMA launched an ERA concept note consultation against some targeted issues prior to an updating the current ERA guidance for medicinal products in 2017. This presentation will describe the EMA ERA guiadance in detail and address some of the key scientific issues associated with the current EMA ERA guidance, EMA concept note and proposed ERA guileline revision drawing from experiences gathered over the past ten years. All obervations and recommendations will be made using empirical data generated to support marketing applications. This includes but is not limited to (i) the scientific basis for the EMA Phase I action limit currently set at 10 ng/l for APIs with no mode of action concerns, and (ii) the Log Kow trigger for the Phase I and Phase II persistence, bioaccumulation and toxicity (PBT) assessments. A thorough review of all available data generated during the ten years that the EMA guidance for the ERA of human medicinal products has been in place is essential in order to improve the existing ERA guidance. Triggers for ERA and PBT assessment can be refined without compromising environmental protection. Approaches to environmental exposure assessment can also be refined (data not included within the extended abstract) as most existing risks assessments are based on worst case predicted environmental concentrations (PECs) that assume no patient metabolism or sewage treatment removal.

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Using HPLC stationary phases to predict fate and behavior of non-ionic and anionic surfactants

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Anionic and non-ionic surfactants are large-scale produced chemicals which are present in many consumer products and consequently also in the environment as organic contaminants. The octanol-water partition constant (K_{ow})is widely used as a hydrophobicity parameter in quantitative structure-activity relationships to predict the environmental fate, behavior and effects of neutral organic compounds. However, K_{ow} is not meaningful for (ionic) surface-active compounds because environmental properties of surfactants are not solely driven by their hydrophobicity. Our research is focused on the development and testing of new approaches and parameters that can be applied in models to predict sorption and bioaccumulation of different surfactant classes. We used measured retention times of selected surfactants on different stationary phases in liquid chromatography (e.g., C_{18}, HILIC or weak ion-exchange phases) to study the three basic sorptive interactions of surfactants (i.e., hydrophobicity, hydrogen-bonding and electrostatic interactions). From the resulting retention times, a logarithmic capacity factor could be calculated that represented the specific surfactant interaction properties: k'_{C18} for hydrophobicity, k'_{HILIC} for hydrogen-bonding interactions, and k'_{WAX} for electrostatic interactions. The logarithmic capacity factors determined for alcohol ethoxylates with both C_{18} and HILIC phases were used in combination with environmental data from experiments or literature (i.e., bioconcentration, toxicity, sorption to clay minerals, or partitioning to membranes and dissolved organic carbon) in a regression model with multiple parameters to determine the contributions of the hydrophobic and the hydrogen-bonding components to their environmental properties. While this approach still requires additional work to model the environmental behavior of anionic surfactants, it seems to work very well for non-ionic surfactants. The possibility of using this method to generate new parameters that can better predict environmental properties of surfactants will have important consequences for the regulatory evaluation of surfactants within REACH and OSPAR.

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Quantifying the influence of technological and spatial variability on the removal efficiencies of chemicals in activated sludge treatment processes

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The use of pharmaceuticals and personal care products leads to chemical emissions in wastewater. In many countries, wastewater is treated in wastewater treatment plants (WWTPs) prior to its release in the environment. However, some of the pharmaceuticals and chemicals contained in personal care products are only incompletely removed upon treatment. When discharged to freshwater, they then represent a potential risk for human health and the environment. Models exist that predict the removal efficiencies (REs) of such compounds by WWTPs using the physicochemical properties of the chemicals. A good understanding of the influence of design parameters of WWTPs on the REs of those chemicals is however lacking. We reviewed literature on studies quantifying the REs of fragrances, surfactants, and pharmaceuticals and used meta regression models to estimate variations in the REs of those chemicals in activated sludge WWTPs due to technological and spatial parameters as well as the physicochemical properties of the compounds. The effect-size of the meta-analysis was defined as the logit transformation of the REs weighted by the number of water sample taken. The following potential moderators were tested to explain the REs of fragrances and surfactants jointly: the type of treatment, the hydraulic retention time (HRT), the sludge retention time (SRT), the inflow rate, the plant capacity, and the influent concentrations, K_{OW}, and K_{OC}. Collinearity and heterogeneity tests led to the choice of the K_{OW}, the SRT, and the influent concentration as moderators. The Akaike Information Criterion (AIC) pointed towards the model including all three moderators as being the best. However, this model had a very large confidence interval explained by the interactions between the K_{OW} values and the type of chemical (fragrance or surfactant). These preliminary results highlight the need for a moderator variable distinguishing between the chemical type, e.g. the biodegradation potential. Also, adding additional plant-specific parameters like pH or the share of domestic wastewater might improve the performance of the model. Further work will focus on incorporating pharmaceuticals in the REs' predictions.

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Mapping risk assessment challenges for HPC ingredients: a chemical space analysis

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The inclusion of chemical ingredients in Home and Personal Care (HPC) products is based on the functionality that their individual physicochemical properties bring towards enhancing the overall performance of the product. Given the broad range of functions that HPC products provide (cleansing, moisturizing, conditioning, etc), chemical ingredients used in these products can therefore capture a broad range of chemical classes, for instance from being extremely hydrophilic to extremely hydrophobic, neutral organics, inorganics, ionisable, and permanently charged salts. As it happens, not only do the physicochemical properties of various chemical ingredients influence the functionality of a HPC product, they also influence the behaviour and fate in test systems (*in vitro*, *in vivo*, and *in silico*) and in the environment. It is thus critical that the physicochemical properties be considered in both experimental design and selection of appropriate in silico tools. Using an example from industry, we examine the chemical space of a selection of HPC ingredients (>7000) and discuss implications towards assessing behaviour *in vitro*. We base our analysis on batch estimates of chemical properties (using SMILES strings), and discuss the validity of such estimates for the chemicals in question. We filter the chemicals using a set of basic criteria to identify groups of chemicals for which standard risk assessment procedures are not applicable. This analysis provides a comprehensive overview of the specific modelling and laboratory research challenges that risk assessors face in dealing with HPC ingredients.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (II)

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Accumulation of cadmium in the brown crab Cancer pagurus from different routes by tracing multiple stable Cd isotopes

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A laboratory experiment was conducted to investigate the main uptake route and accumulation of cadmium in the edible crab. By tracing two different isotopes, it is feasible to survey two different uptake pathways simultaneously in the same animal, minimizing the number of crabs used. ICP-MS QQQ measurements with a low detection limit gave us the opportunity to use low concentrations with 500 ng/L 106Cd in seawater and 1 mg/kg wet weight 108Cd in feed. We used gavage feeding to standardize the feeding of the crabs (78 in control and 78 in the treatment group) to 2 mL per feeding and three times a week. Five crabs were dissected at each of the sampling days in the exposure phase (0, 2, 7, 14, 21, 30 and 42) and the depuration phase (2, 4, 7, 21, 35). We analyzed hepatopancreas, gills and hæmolymph for both isotopes. Uptake and elimination kinetics will be described applying a compartmental modelling and assuming first order kinetics. This knowledge is desired because of high concentrations of cadmium from unknown origin in edible crabs in different parts of Europe and especially Northern Norway.

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Use of reverse-isotopic labeling of Lymnaea stagnalis to understand dietborne Cu bioavailability in receiving waters affected by contamination and remediation of acid mine drainage

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As part of a project to evaluate remediation of acid mine drainage in the North Fork of Clear Creek (NFCC) in central Colorado, USA, we traced the flux of Cu in isotopically-labeled freshwater snails (*Lymnaea stagnalis*) that were fed metal-rich material collected from the sediment. The isotopic signature of the snails was reversed by culturing them in a sub-lethal concentration of ^{65}Cu-enriched water for five weeks. Various amounts of metal-contaminated material were mixed with diatoms (*Nitzschia palea*) to produce a series of dietborne-metal concentrations, and each mixture was collected on a filter to create a mat the snails ate. Using mass balance and isotopic-ratio conversions, uptake and depuration of Cu was traced during a 48-h feeding exposure. We evaluated biodynamics of three types of metal-contaminated particles: (1) loosely-coated floc, (2) armored coating on the rocks, and (3) a mixture of the floc and armored coating. The uptake rate of Cu was greatest from the loosely-coated floc, followed by the mixture of loose and armored material and the armored coating alone. However, the Cu assimilation efficiency, which is an index of the bioavailability of metals, remained approximately 40-50% and did not differ significantly among the three types of metal-rich material. Based on these data, we conclude that the that the physical form of metal-contaminated food could be an important factor for determining the bioavailability of Cu, and we hypothesize that the physical form could change when scheduled remediation of NFCC begins in January 2017. Ongoing research is underway to characterize physical changes of the sediment coatings and bioavailability throughout the remediation process.

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From biodynamic modelling to the field: Cd and Cu availability for invertebrates in river floodplains

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Bioavailability is accepted as a key concept allowing to quantitatively relate the changes in the trace metal concentrations and speciation with the intensity of the induced biological effects. In the specific case of invertebrates, different models were developed explain bioavailability ranging from models that consider uptake from water only to biodynamics, which consider different uptakes routes. However, the application of these lab models with model invertebrates to the field as well as the transferability of uptake and loss parameters to species other than the model species is still to explore. The objective of this study is to test the applicability of the biodynamic model to predict the whole body concentrations, as a measure for bioavailability, in invertebrates at a larger scale in the field. The specific focus is on floodplain water bodies as they present a diverse invertebrate community and a variety of physico-chemical conditions. To elucidate the quantitative link between environmental trace metal concentrations, speciation and whole body metal concentrations in invertebrates, a biodynamic modelling approach was adapted to field conditions with the integration of novel input parameters for concentrations in water and food. Cu and Cd concentrations in water, sediment, SPM, diffusive gradients in thin films (DGT) concentrations in water in sediments and water quality variables were measured and used as input parameters. Cu and Cd in model invertebrate species, mayfly species, gastropods and amphipods sampled in the 6 different flood plains of Rhone River, characterized by different degree of connection with the main channel: three permanently connected secondary channels and three permanently disconnected floodplain water bodies were measured. The modelled values were compared to measured whole body concentrations values and the difference was used to evaluate the model performance. The obtained results demonstrated that the accumulation of Cu and Cd by aquatic invertebrates in the field can be predicted by biodynamic modeling using the constants obtained from the lab experiments. Labile concentrations measured with DGT in sediment and water are promising input variables for the biodynamic model. Food is the main uptake route when uptake is modeled with the biodynamic model.

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Are zinc sacrificial anodes a major source of zinc to the estuarine environment: A case study of the Hamble, UK

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Sacrificial zinc anodes are used on millions of vessels worldwide to provide cathodic protection to metal components. Zinc anodes are generally not considered a major source of zinc to estuaries, compared with other sources such as waste water treatment works. However water and sediment sample collection on the Hamble estuary, which has 3,000 pleasure vessels indicates zinc anodes could be the main source to the estuary. Antifouling paints will also contribute zinc to the estuarine environment from pleasure craft, this is however likely to be small compared to anodes. Annual corrosion rates suggest up to 6.95t/yr of zinc is input into the Hamble from this source. The corrosion rate was calculated using annual corrosion per vessel and anode weights from a survey of boat owners. Similar estimates for wastewater treatment works suggest 0.062 to 0.151t/yr of zinc is input into the estuary, which is considerably lower than anode inputs. The MAMPEC model was also used to predict zinc levels released from anodes, which predicted similar levels to those observed on the Hamble. Water samples were analysed using cathodic stripping voltammetry and data shows that zinc anodes and other zinc sources are currently causing the Hamble to exceed the EQS of 7.9μg/l. Observed total dissolved zinc concentrations ranged between 3 and 35μg/l, with the majority of samples between 5 and 20μg/l. Concentrations above the boat moorings and in the Hamble river itself, were measured to be below 8μg/l suggesting the river to be a minor source of zinc to the estuary. Two freshwater samples from the Hamble showed total dissolved zinc concentrations of 6.41 and 4.23μg/l indicating the riverine input to be relatively low. These observations suggest zinc anodes are a major source of zinc to the Hamble estuary. Sediment samples were oven-dried and analysed using a portable XRF. Zinc concentrations in sediment samples ranged between 84 and 226μg/g. Sediments did not show any significant correlation with boat numbers, unlike the water samples suggesting dissolution of zinc from anodes largely impacts the dissolved rather than particulate phase.

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Distribution of Trace Metals (Cu, Pb, Ni, Zn) between Particulate, Colloidal and Truly Dissolved Fractions in Wastewater Treatment

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Water companies are facing increasing pressure to remove metals from wastewater treatment effluents in order to comply with Environment Quality Standards. Metal removal within traditional wastewater treatment processes is, however, variable and is leading water companies to consider the deployment of expensive and energy/carbon intensive tertiary treatment options. The form of metal within the effluent matrix is thought to play a role in controlling the extent to which these are removed and a better understanding of the metal form will enable the development of effective treatment technologies. In this study, the distribution of Cu, Pb, Ni and Zn particle size fractions in wastewater from a trickling filter treatment works was investigated. Samples of influent, primary effluent, humus effluent and final effluent were collected and separated into particulate (i.e. > 0.45 μm), colloidal (i.e. 1 kDa to 0.45 μm), and truly dissolved (i.e. < 1 kDa) fractions using membrane filters. In the influent, substantial proportions of Cu (60%), Pb (67%), and Zn (32%) were present in the particulate fraction which was removed in conjunction with suspended particles at the works in subsequent treatment stages. In final effluent, sizeable proportions of Cu (52%), Pb (32%), Ni (44%) and Zn (68%) were found within the colloidal size fraction. Calculated ratios of soluble metal to organic carbon suggest the metal to be adsorbed to or complexed with non-humic macromolecules typically found within the colloidal size range. These findings indicate that technologies capable of removing particles within the colloidal fraction have good potential to enhance metals removal from wastewater. \n

Modelling and monitoring of pesticides fate and exposure in a regulatory context (I)

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Not relevant? The significance of non-regulated pesticide metabolites
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Non-relevant metabolites are formation products from pesticides, which were characterized as not biologically active or toxic. There are no legal thresholds for groundwater concentrations in plant protection regulation so far, which means high uncertainties for environmental risk assessment. Water supplying companies reported about problems with non-relevant metabolite findings, which cause higher processing costs and decreasing acceptance by costumers. In Germany there is a common understanding that a threshold for non-relevant metabolites in groundwater protection law is necessary. It is discussed how pesticide regulation can contribute to the minimization of non-relevant metabolite leaching. To capture the extent of groundwater contamination with non-relevant metabolites in Germany two datasets from federal pesticide monitoring programmes were evaluated. Many of the existing non-relevant metabolites are still not part of the monitoring programmes, and the ones considered were only included few years ago. Obviously the issue is of rising interest, but the story will complete only gradually with open questions remaining. In 45% of the monitoring wells non-relevant metabolites were found above the detection limit, in 35% above 0.1 $\mu g\ L^{-1}$ and in 13% above 1 $\mu g\ L^{-1}$. Therefore elevated concentrations of those are a mass-phenomenon in Germany. However, the most frequent and highest detects derive from the same few active substances. Considering all detects above 0.1 $\mu g\ L^{-1}$ there are only 13 parent substances, detects with values above 1 $\mu g\ L^{-1}$ comprise only 8 parent substances, three of which with expired or expiring approval. The low number of active substances concerned makes risk management a promising option to solve the majority of issues with a minimum effort. A regulatory management option for prevention of non-relevant metabolite contamination was just implemented in Germany (NG301). It enables restrictions of respective parent substances for drinking water abstraction areas with proved elevated concentrations of non-relevant metabolites. Independent of regulatory action there are approaches by water supplying companies, who support the research on and use of alternative plant protection products with lower leaching potential. These initatives are highly appreciated, but should be extended to all aquifers and not be limited to drinking water abstraction areas.

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Significant processes for calculating environmental concentrations of plant protection products in the sediment compartment of FOCUSsw scenarios
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Predicted environmental concentration in sediment (PECsed) of plant protection products (PPP) for the EU regulatory purposes is currently calculated according to the FOCUS surface water report. This currently considers periods of 12-16 months for the exposure assessment in surface water bodies. However, in a recent scientific opinion of the European Food Safety Authority (EFSA) it is argued that the accumulation of PPP in sediments over significantly longer time periods may be a relevant process. Therefore, the EFSA proposed to introduce a sediment accumulation factor in order to account for enrichment of PPP substances over several years in the sediment. The calculation of this accumulation factor, however, would consider degradation in sediment as the only dissipation route, and would not take into account sediment dynamics or desorption back into the water column. In order to assess transport and deposition dynamics of suspended sediment on the possible extent of substance accumulation in the sediment phase, the hydraulic model HEC-RAS was employed. The model was parameterized according to the stream characteristics of the FOCUS scenarios and was run over a period of 20 years. The results show that for the distribution of grain sizes and the ranges of flow velocity in the FOCUS stream scenarios only about 10% to 60% of the incoming sediment would be deposited at the stream bed, while the remaining portion of sediment particles would be transported across the downstream boundary. For a generic PPP substance this resulted in a deposition of only about 20% to 50% of incoming PPP substance at the river bed. In a separate analysis, the FOCUS TOXSWA model was utilised to examine the relative importance of degradation versus desorption from the sediment compartment. Across shorter [1 – 1.5 year] and longer term simulations [20 years] for a range of generic PPPs it was demonstrated that desorption and back-diffusion to the water phase was generally the dominant dissipation process from the sediment compartment. The results of this study show that sediment dynamics and desorption should be considered for the calculation of PECsed across longer time scales. Neglecting to account for these processes leads to overestimation of the potential for accumulation in the sediment.

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Use of Pesticide Groundwater Monitoring data as higher tier for regulatory risk assessment
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In the European regulatory framework risk assessment is mandatory performed for pesticides. No unacceptable risk of groundwater contamination has to be proven according to Regulation 1107/2009. Routinely, Predicted Environmental Concentrations in Groundwater (PECgw) are derived using numerical models for active substances and metabolites. In addition to modelling, the European regulatory framework also allows for Groundwater Monitoring data to be used for pesticides approval. In the tiered approach, Groundwater Monitoring data are identified as higher tier that may supersede the modelling. Still, there is currently no European guidance available on the use of groundwater monitoring data for regulatory purposes. In France, Groundwater Monitoring data have been submitted to Anses (French Agency for Food, Environmental and Occupational Health & Safety) in order to support the renewal of pesticides. The aim of this presentation is to provide a feedback based on different worked examples. These feedbacks include focus on the main issues that were identified and the general recommendations that were made when targeted monitoring were submitted. The main issues when dealing with Groundwater Monitoring data interpretation were related to site selections and related vulnerably, and then of how representative was the Groundwater monitoring. Recommendations were made to address both issues and will be presented. In addition, proposal to combine targeted together with public Groundwater Monitoring dataset were made to enhance the representativeness of the GW monitoring conducted and will also be presented. \n

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The potential of 'on-line' constructed wetlands for mitigating pesticide transfers from agricultural land to surface waters
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Pesticides make important contributions to modern agriculture but losses from land to water can present problems for environmental management, particularly in catchments where surface waters are abstracted for drinking water. Where artificial field drains represent a dominant pathway for pesticide transfers, buffer zones provide little mitigation potential. Instead, “on-line” constructed wetlands have been proposed as a potential means of reducing pesticide fluxes in drainage ditches and headwater streams. Here, we evaluate the potential of small free-surface wetlands to reduce pesticide concentrations in surface waters using a combination of field monitoring and numerical modelling. Two small constructed wetland systems in a first order catchment in Cambridgeshire, UK, were monitored over the 2014-2015 winter season to evaluate the fate of metaldehyde, a commonly-used molluscicide. Metaldehyde has been regularly detected at high concentrations in surface water samples in a number of drinking water supply catchments in the UK and unusually difficult to remove via conventional drinking water treatment. Metaldehyde losses from the upstream catchment were significant with peak concentrations occurring in the first storm events in early autumn, soon after application. Concentrations and loads appeared to be unaffected by transit through the wetland over a range of flow conditions – probably due to short solute residence times (quantified via several tracing experiments). A dynamic model, based on fugacity concepts, was constructed to describe chemical fate in the wetland system. The model was used to evaluate mitigation potential and management options under field conditions and for a range of different pesticides under alternative flow and wetland dimension scenarios. Scenario analysis suggested that, even for pesticides with relatively short aquatic half lives, wetland systems would need to be much larger than those studied here in order to get any appreciable attenuation.

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Evaluation of Multi-year FOCUS Surface Water Calculations for Regulatory Purposes
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In the regulatory risk assessment of pesticides in Europe aquatic exposure concentrations are calculated with the FOCUS Surface Water scenarios, using weather data for fixed 16-months modelling periods that are pre-selected for each scenario. These pre-selected modeling periods have recently been criticized for not consistently representing a realistic worst case exposure situation. It is also unclear if the resulting exposure patterns are suitable for use in higher-tier risk assessments

that are based on details of exposure peak characteristics. \n One option to address these issues is to extend the modelling period and calculate aquatic exposure over multiple years with annual application. This allows putting the exposure peaks and patterns of the standard FOCUS scenarios into the context of variable weather conditions which can provide a more solid basis for risk assessment. While FOCUS Surface Water multi-year exposure calculations are technically feasible, interpretation of the results and the use of multi-year exposure data in (higher-tier) risk assessments is still under discussion. \n In this presentation we investigate 20-year predicted aquatic exposure data for different example compounds and compare a range of options for their regulatory interpretation. Since the exposure patterns are strongly driven by extreme weather conditions with unknown return periods, it was necessary to interpret them in the context of long-term weather data. We discuss the selection of appropriate peak exposures percentiles for an overall realistic worst case risk assessment, and the need to address separately special cases in which the worst case exposure significantly exceeds the selected percentile. Other options include the identification of realistic worst case exposure patterns for comparison with ecotoxicological studies, and the application of ecotoxicological effect models to selected or multi-year exposure patterns.

Nanomaterial fate and toxicity - Implications of the environment as a global reactor for nanomaterials along their life-cycle (I)

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Toxicokinetics of silver nanoparticles in the freshwater snail Physa acuta

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The emergent demand brought by the nanotechnology field has led to the large-scale production and commercialisation of nanoparticles (NPs), resulting in their potential release into the environment, with aquatic ecosystems being an important final sink. Risk assessment needs refinement especially for NPs, due to their complex behaviour, properties and interactions with environmental factors and organisms. Understanding the bioaccumulation and distribution of contaminants in organisms is considered essential for environmental risk prediction. Toxicokinetics studies can provide answers to these challenges. The aim of the present work was to assess the uptake and elimination kinetics of different silver nanoparticles (Ag-NPs), one of the most manufactured NPs, in the freshwater snail *Physa acuta*. Four different Ag-NPs (3-8nm, 50nm and 60nm), including one silver sulphide NP (Ag_2S-NPs), and their ionic counterpart as silver nitrate ($AgNO_3$) were tested. The organisms were individually exposed for 7 days to the test compounds at a nominal concentration of 10 μg Ag.L^{-1} and then transferred to clean media for the 7-day elimination phase. At different points in time, animals were sampled and analysed for body Ag concentrations by graphite furnace atomic absorption spectrometry. Kinetics of Ag-NPs and ionic Ag in the snails were described by a one-compartment model. The results revealed differences in the uptake and elimination kinetics of the different Ag forms in *P. acuta*. Snails exposed to 60nm Ag-NPs and ionic Ag displayed higher uptake rate constant (k1) values than those exposed to smaller Ag-NPs. These organisms also presented higher elimination rate constant (k2) values when compared to the animals exposed to the other Ag forms. Very little or no Ag elimination was observed from the organisms exposed to 3-8nm Ag-NPs and ionic Ag during the 7-day elimination phase. Ionic Ag presented an uptake rate similar to that of the larger Ag-NPs but the depuration kinetics was more similar to that of the smaller Ag-NPs. This may denote alterations in the behaviour of these compounds in solution and/or inside the organism. Furthermore, the very slow elimination upon exposure to small Ag-NPs and ionic Ag ($AgNO_3$) could lead to the potential transfer of Ag to higher trophic levels in the aquatic ecosystem.

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Toxicokinetics of silver nanoparticles in the mealworm Tenebrio molitor

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By using nanoparticles (NPs) in several applications, exposure is inevitable. Soil, as a substrate at the basis of many food chains, may especially be exposed, e.g. through sewage sludge application onto the agricultural field, with AgNPs as the most abundant NP type. To assess the risk of AgNPs, knowledge on the bioaccumulation of different forms of AgNPs in different terrestrial organisms is essential. The aim of this work was to determine the toxicokinetics of different AgNPs in mealworms. The soil was spiked with 100 mg Ag/kg dry soil with different silver AgNPs (3-8 nm, 60 nm, 50 nm, Ag_2S) and $AgNO_3$. Mealworms were exposed to the contaminated soil for a 21-day uptake phase and then transferred to clean soil for a 21-day elimination phase. Mealworms were collected at each sampling point (1, 3, 6, 9, 15, 21 days) for both phases. At each sampling time, animals and their molt (if they molted) were frozen individually (-20?C), dried, digested and analysed for Ag by graphite furnace Atomic Absorption Spectrophotometry. Two one-compartment models with two stages were used to calculate the Ag uptake and elimination kinetics. In this study, no significant difference was seen among the toxicokinetics in mealworms exposed to different forms of silver, with uptake rate constant (k1) values of 0.23, 0.38 and, 0.30 g soil/g animal/day and elimination rate constant (k2) values of 1.06, 1.04 and 1.17 day^{-1} for NP3-8nm, NP60nm and Ag^+ respectively. Bioaccumulation factors (BAF) showed that AgNP60 were more accumulated in the mealworms compared to AgNP3-8nm and Ag^+. Moreover, based on the estimated inert fractions (0.32, 0.14, and 0.37 for NP3-8nm, NP60nm and Ag^+, respectively) the AgNP60nm were less retained by the mealworms compared to other studied Ag forms, while Ag^+ and AgNP3-8nm displayed an insignificant difference. This may be due to higher Ag^+ release from smaller silver nanoparticles. The analysis of the molts of the mealworms showed that the amount of silver which can be eliminated by molting is insignificant. Toxicokinetics in mealworms exposed to Ag NPs and ions were similar showing a high ability of the organisms to eliminate Ag after being transferred to clean soil. This indicates that mealworms do not store Ag as observed for other soil organisms (e.g. isopods). This study showed that the bioaccumulaton of different forms of NPs, which can also simulate their aging, should be considered in risk assessment of NPs. Ag_2S uptake still has to be determined.

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Toxicokinetic rate constants of particulate Ag and Ag+ differ in Eisenia fetida exposed under environmentally relevant conditions

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The soil represents an important environmental compartment that can be regarded as a final sink for silver nanoparticles (Ag-NPs). Disposal of sewage sludge onto agricultural fields is a relevant source of Ag-NPs in terrestrial environments which can lead to risks for soil organisms. Assessing realistic exposure scenarios and bioavailability of Ag-NPs for soil organisms is a challenge since Ag-NPs can undergo numerous physico-chemical transformations in soil and within organisms. Moreover, the detection of NPs in complex matrix is hampered due to the lack of straightforward analytical methods. Most of the ecotoxicological studies on uptake of metal NPs in organisms quantified NPs by analysis of total metal content. To our knowledge this work is the first *in vivo* Ag-NPs toxicokinetic study on *Eisenia fetida* that characterises and quantifies uptake and excretion of ionic as well as particulate Ag under environmentally relevant conditions. Earthworms were exposed to 15 mg Ag Kg^{-1} soil of Ag-NPs (50 nm) and $AgNO_3$. Every 7 days, throughout 28 days of exposure followed by 28 days of depuration in clean soil, concentrations of both Ag-NPs and Ag^+ were quantified in earthworms tissues, faeces and soil by ICP-MS and sp-ICP-MS. Also NPs size distributions were assessed. After 28 days of exposure Ag concentration in earthworms was 4.0 mg Ag Kg^{-1} wet body weight in particulate form and 9.5 mg Ag Kg^{-1} wet body weight as total Ag content. Ag-NPs concentrations in faeces were relatively constant over the exposure time, in average 0.96 mg Ag-NP Kg^{-1}. Finally a first-order one-compartment model was applied to calculate initial Ag-NPs and Ag^+ uptake (K_1) and elimination (K_2) kinetics parameters: K_1 is 0.015 and 0.039 g_{soil} g^{-1}_{animal} day^{-1}; K_2 is 0.070 and 0.048 day^{-1}, for Ag-NPs and Ag^+ respectively. This is the first study that provides kinetic constants for worms based on detailed NPs concentrations and not just on total concentrations. The differences between kinetic rates of Ag-NPs and Ag^+ in earthworms exposed under environmentally relevant conditions underline the need to address both NP and ions transformations in exposure media and within organisms in order to predict their fate and their hazard. This study shows that read-across of kinetic constants between different forms of the same material may not be straightforward at all.

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Exposure pathway depended impacts of silver and titanium dioxide nanoparticles on Gammarus fossarum

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Apart from their economic value, nanoparticles (NPs) pose a potential risk for the integrity of aquatic ecosystems. Due to their broad range of applications and frequent use, a release into surface waters seems inevitable. Given their tonnage, titanium dioxide ($nTiO_2$) and silver (nAg) NPs are among the most important. Numerous investigations indicate sediments as the final sink, leading to an increase in exposure of benthic species. Although reports of sub-lethal effects of NPs on benthic species are increasing, the importance of the exposure route – either waterborne or dietary – is poorly understood. The purpose of this study was to uncover the influence of these exposure pathways on the effects caused by $nTiO_2$ and nAg. Therefore, for each NP type an individual 30-d long bioassay with the benthic amphipod shredder *Gammarus fossarum* was conducted, using a 2×2 factorial test design. The factors comprised of the presence or absence of the NP ($nTiO_2$: ~85 nm, 4 mg/L or nAg: ~35 nm, 0.125 mg/L, respectively) in the water phase, combined with NP presence or absence during a preceding 6-d long aging of the provided food (black alder leaf material). The endpoints investigated during the course of the experiments were mortality, leaf consumption, feces production and assimilation. Furthermore, after the termination of the experiment the physiological fitness was examined by using dry weight and lipid content as proxies. Results revealed a significantly reduced assimilation induced by waterborne exposure towards $nTiO_2$ (up to 30%). In contrast, the dietary exposure towards nAg significantly increased the organisms' assimilation (up to 50%). For nAg the combination of both exposure pathways resulted in a distinct mortality of ~30%, whereas in the control treatment all organisms survived. Dry weight and lipid content were not significantly affected, regardless of the NP type and exposure scenario. This study suggests that the influence of NP exposure pathways cannot be generalized and remains particle specific, as for the two investigated NP types the direction of observed effects varied substantially, and moreover was dependent on the route of exposure.

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Routes of uptake and bioaccumulation of cerium oxide and silver nanoparticles depend on their fate in sediments

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Engineered nanoparticles (NPs) undergo myriad transformations upon entering the aquatic environment, depending not only upon the composition of the particles but also the physicochemical properties of the surrounding media. Sediments are predicted to be a major sink of NPs released into the aquatic environment due to processes of aggregation and sedimentation occurring over relatively short timescales of a matter of hours to days in freshwaters. What is less understood is if NPs entering sediments will have the same capacity for toxicity as particles in suspension in the overlying water? Will transformations in sediments reduce or enhance nanoparticle bioavailability? Will these transformations be the same for NPs of different core or surface properties? This work aims to address some of these questions. Specifically we hypothesise that the persistence of dissolution products or a colloidal fraction of NPs in sediment pore waters will allow for transdermal uptake of NPs into sediment dwelling species. We generated two viable phenotypes of the sediment dwelling aquatic worm, *Lumbriculus variegatus* through fragmentation to produce feeding and non-feeding worms. These were exposed to cerium oxide (CeO_2- NPs) or silver nanoparticles (Ag NPs) stabilised either electrostatically with citrate or sterically with PEG (mono mPEG phosphonic acid ester). A combination of centrifugation and ultrafiltration techniques were employed alongside the biological exposures to examine the fate of nanoparticles in the sediment pore waters, quantifying the colloidal fraction containing CeO_2or Ag NPs (Results indicate that NPs which associate with the solid fraction of the sediment and do not dissolve within the sediments (CeO_2 NPs) were only available through ingestion. These particles were also less bioavailable than Ag NPs and neither their fate nor accumulation differed between the two forms of stabilisation. PEG coated Ag NPs experienced significantly greater accumulation of Ag through transdermal uptake than either Citrate-Ag or silver nitrate and not all of this transdermal uptake of Ag could be accounted for by dissolution. The cause for this is the focus of ongoing experiments to be discussed in more detail during the presentation, alongside ongoing investigations into Ag NP transformations during the gut transition in these aquatic worms and their kinetic uptake from sediments over time.

Poly- and perfluoroalkyl substances (PFASs): Recent developments, sources, transport, fate and toxicity (II)

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The burden of persistence: towards an understanding of ΣPFAS exposure

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The US EPA recently published a Drinking Water Health Advisory for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) at a combined concentration of 0.07 ppb. The New Jersey Department of Environmental Protection recommends a drinking water guidance level of 0.04 ppb, based on a lifetime (70 year) exposure. The concept of lifetime exposure is very relevant for these chemicals because they are extremely persistent. At the same time, a wide variety of other per- and polyfluorinated alkyl substances (PFAS) are used in applications ranging from industrial processing aids to personal care products, often as replacements for long-chain acids. Their properties are largely unknown. Given the increasing presence of mixtures of these substances in the environment, an important question is whether guidance levels should be expanded to include other PFAS and other routes of exposure. Here, we make a first step towards understanding total PFAS exposure by addressing three questions: (1) How much is known about their occurrence in key matrices? (2) How can we estimate the combined exposure of populations in different geographical regions to PFAS? (3) Can we use current information on their toxicity and biological fate to classifiy potential impacts of novel PFAS? Our results indicate that drinking water levels in most regions exceed guidance levels and that few studies quantify concentrations for PFAS other than PFOA and PFOS, even in recent publications. A similar over-representation of PFOA and PFOS also exists in the toxicological literature, and there is wide variance in toxic effect concentrations and endpoints reported. The disruption of lipid metabolism, with a resulting cascade of impacts, is a common effect across species and assays types. The picture for novel PFAS is less clear,but does not inspire confidence. Our molecular modeling results indicate that their interaction with key biological macromolecules is driven by the total number of perfluorinated carbons in the molecule. Recent observations of their persistence and bioaccumulation potential appear to bear this out. Given the extreme persistence and high bioavailability of PFAS, it is critical that we develop more refined understanding of their combined toxic impacts. Given known toxicity of legacy PFAS like PFOA and PFOS and high uncertainty in toxicity of novel PFAS, a truly precautionary response suggests emissions of such extremely persistent substances cannot be allowed.

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The environmental emissions, global distribution and risk assessment of F-53B

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The environmental releases and concentrations of 6:2 chlorinated polyfluorinated ether sulfonate (6:2 Cl-PFAES, with the trade name F-53B) as a Chinese specific alternative of perfluorooctane sulfonate acid (PFOS) kept increasing recently and F-53B had become one of the main fluoride organic pollutants in some given water mediums, which had been subjected to incremental public scrutiny. In this study, the annual F-53B releases in China were estimated at approximately 10~14 t during the period of 2006-2015 based on the bottom-up emission estimation method and the analysis of statistic data from Chinese metal plating sector. A global fate model of Persistent Organic Pollutants (Globo-POP) was used to assess the global distribution and long-range transport of F-53B by presenting the Arctic Contamination Potential (ACP) and the intercompartmental and meridional transfer rate. The model output demonstrated that F-53B could be transported from source locations to remote regions with moderate efficiency and only a small fraction of total F-53B emission reached the Arctic, of which almost all was transferred by ocean. The distributed pattern of F-53B was similar to PFOS probably due to the slight differences of partitioning coefficients. Then the results of a risk assessment model (EUSES model) indicated that the potential threat to environmental and human health couldn't be ignored with the increasing usage and emission of F-53B, especially in local scale in China. Considering the properties of PBT, we reckoned that the application of F-53B as an alternative of PFOS couldn't reduce the risk and it should be listed as a regrettable substitution.

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Degradation of poly- and perfluoroalkyl substances (PFASs) from industrial wastewaters on BDD electrodes

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Electrochemical oxidation is a promising technology for the degradation of highly persistent per- and polyfluorinated compounds (PFASs). However, so far the efficiency of electrochemical processes to degrade PFASs has been mostly studied with model solutions of single compounds such as PFOA and PFOS [1]. The present work aims to study, for the first time to our knowledge, the electrochemical removal of PFASs from the effluent of an industrial wastewater treatment plant using a commercial boron-doped diamond (BDD) electrode. The degradation of 29 PFASs was assessed, together with the evolution of total organic carbon (TOC) and principal anions. The group of PFASs that were monitored included perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids, n:2 fluorotelomer carboxylic acids, n:2 unsaturated fluorotelomer carboxylic acids, n:2 fluorotelomer sulfonic acids, perfluorooctane sulfonamide, 6:2 fluorotelomer sulfonamide alkylbetaine and 6:2 fluorotelomer sulfonamide propyl N.N dimethylamine [2]. The experimental results evidenced the efficacy of the electrochemical technology for the degradation of the PFASs contained in heavily contaminated real wastewaters. The initial SPFASs concentration of 1654 mg/L was reduced at a rate of 99.7% in the treated effluent by applying a constant current density of 50 mA/cm^2 for 10 hours of operation. Furthermore, the TOC removal rate was 91%. It was found that the major fluorotelomers contained in the feed were initially converted into perfluorohexanoic acid, which nevertheless was also degraded into shorter chain PFCAs. A defluorination factor of 126% elucidated that other unknown PFASs were being degraded during the electrochemical treatment of the wastewater.

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Development and assessment of alternatives to long-chain PFAS
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The family of perfluoroalkyl substances (PFAS) comprises many compounds manufactured for several decades and used in a variety of applications ranging from protective garments, to airplane hoses, electronic equipment and fire-fighting agents. Their high stability and ability to resist extreme environmental conditions makes them not only ideal compounds used to protect man and equipment, but also raises some issues related to their overall persistency in the environment. Some PFASs have been classified as toxic and bioaccumulative, and are regulated by international regulatory bodies, or are currently being assessed for future regulations at regional or national level. The major EU, US and Japanese fluorotechnology manufacturers have over the last decade developed alternatives to a specific class of per- and poylfluorinated compounds referred to as long-chain PFAS. These alternatives were developed under the umbrella of the "2010-2015 EPA Product Stewardship Program", a global voluntary agreement signed between the major fluorotechnology manufacturers and the US Environmental Protection Agency. The goal of developing these alternatives was to provide chemicals with a reduced environmental impact while retaining the same performance as the long-chain PFASs. This presentation presents an overview of the processes required to develop alternatives with an emphasis on the assessment requirements that have to be taken into consideration when developing more sustainable products that are essential to many modern life applications. In addition to assessing these chemicals for their potential PBT properties, other important factors to take into consideration such as performance, exposure and emission controls, and potential remediation procedures will be presented.

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TBD

Input/output and Hybrid Life Cycle Assessment for supporting the assessment of production and consumption patterns

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Hybridizing Ecoinvent and Exiobase with streamlined methods and open-source software
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When done correctly, the hybridization of lifecycle assessment (LCA) and input-output (IO) analyses can combine the detail of the former and the completeness of the latter. However, generating hybrid LCA-IO analyses is data and labour intensive, requires the manipulation of multiple large datasets, forces practitioners to work with both physical and monetary units, and requires a careful correction for so-called double-counting. Multiple hybridization techniques have been proposed, but these are often rather complex and require extensive data on product prices. Hybrid LCA-EEIO analyses remain rare and restricted to individual case studies, rather than reaching mainstream practice or becoming the norm in database development. As databases such as ecoinvent do not have complete cost coverage and systematically underrepresent requirements for services and infrastructures, even studies that hybridize their foreground inventories suffer from truncation when relying on process databases for their background. The present research aims to address these issues. We develop a streamlined hybridization process that is lean on data and is facilitated by a new open-source software, pyLCAIO. This approach is then employed to hybridize not only case studies but also the complete ecoinvent database, combining it with the multiregional IO database, Exiobase. To this end, we address multiple practical and methodological issues, notably pertaining to correction for double-counting and price heterogeneity. Ecoinvent and Exiobase are proving to be highly compatible and complementary databases: both represent value chains spanning international borders in a multiregional manner, both have a strong focus on the energy sectors and on resource use. Their ongoing hybridization should yield (1) an optimally detailed and complete description of the technosphere, (2) global insights into the issue of truncation in LCA practice in different sectors of interest, and (3) a solid foundation for prospective environmental and economic analyses. This analysis of the complementarity between Exiobase and Ecoinvent is already yielding new insights for the optimal allocation of hybridization efforts in individual case study projects.

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Increasing the regional resolution of the Ecoinvent LCI database with GMRIO data
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1. Motivation While in the past, spatial detail has been widely neglected, there is currently a trend in Life Cycle Impact Assessment (LCIA) method development towards more regionalization. For example, the new recommended methods by the UNEP-SETAC life cycle initiative for land use impacts on biodiversity, water scarcity and impacts on human health due to water consumption all apply regionalized characterization factors. The regional resolution of these methods is however not available on the inventory level. Economic data from global multi-regional input-output (GMRIO) databases like Exiobase can be used to increase the regional resolution on the inventory level, which then in turn allows to apply the regionalized LCIA method to capture the difference in impacts. **2. Methods** In this study, we use input-output data from Exiobase to increase the regional resolution of the ecoinvent database. New LCI unit process datasets are created for all regions in Exiobase, based on corresponding dataset in the ecoinvent database. The unit process datasets are matched to their encompassing industry sector using their industry classification. The economic relation between the industry sectors from Exiobase is then employed to re-link the newly created unit process datasets. The described procedure yields a new LCI database with high regional resolution, where economic relations from Exiobase are merged into the structure of the Ecoinvent LCI database. This approach can be considered a Hybrid approach, where GMRIO data is used to estimate international markets and therefore origins in the supply-chain, without changing the detailed, process specific exchanges from ecoinvent. **3. Results and conclusions** The application of the new recommended method for land use and land transformation impacts on biodiversity by Chaudhary et al. (2015) with the regionalized inventory demonstrates two advantages of the presented approach. On one hand, the regionalized supply chains capture differences on the inventory level. And on the other hand, differences on the impact level can be detected consistently. While in the past, regionalized impact assessment has been limited to foreground systems or specific case studies, the presented approach allows quantifying regionalized impacts of any ecoinvent process and its full supply chain.

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EU life cycle indicators - based on hybrid LCA
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Within the work carried out by the Joint Research Centre (JRC) of the European Commission (EC) on Indicators and Assessment of the Environmental Impact of EU consumption, this study focusses on an alternative to the bottom-up LCA modelling adopted so far. The purpose of the study is to calculate consumption based life cycle indicators for the EU28 member countries. The results on the total GHG emissions relating to EU28 are calculated using the multiregional hybrid database Exiobase v3.8.8. Exiobase is created through four EU FP6 and FP7 projects (EXIOPOL, FORWAST, CREEA and DESIRE). The data behind the database includes monetary supply-use tables for 43 countries plus 5 rest-of-world regions, as well as their counterparts in physical mass and energy units. Hence, the database is based on a combination of complete global balanced economic, mass-flow and energy-flow accounts. For the current study, results are calculated both as domestic direct emissions in the EU28 (territorial-based approach) and as emissions relating to final consumption in the EU28. The total GHG emissions calculated using the territorial-based approach in the EU28 are 4.7 billion tonne CO_2-eq. (9.3 tonne CO_2-eq. per capita). Using the consumption-based approach, the results increase by almost 25% to 5.8 billion tonne CO_2-eq. (11.5 tonne CO_2-eq. per capita). This change is because the embodied GHG emissions in imports are higher than in exports. The input-output approach has shown to be a very efficient way to calculate product category, national and regional baselines. The advantage of Exiobase over other input-output databases is that it is a true and fully balanced hybrid model, making it possible to analyse results using physical units of the functional unit and not only monetary. In fact, the database does not look very different from conventional LCA databases such as ecoinvent and ILCD. For JRC's future work with life cycle indicators, when more precise LCA data are needed for specific products, it is recommended to use hybrid input-output data as a starting point, and then adjust these so that the most important flows for the processes/industries of concern are aligned with quality assured traditional process-based life cycle inventories.

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BONSAI: from vision to implementation
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The Big Open Network for Sustainability Assessment Information (BONSAI) is a collaborative effort with the objective of creating the first and most comprehensive global hybrid open-source database for sustainability analysis. BONSAI provides a virtual environment where data are stored, verified and refined by users. The objective of this paper is to summarize the state of the art of the development of the beta version, where *all primary features* are implemented, although the version is not final due to performance or stability issues. The three basic principle of BONSAI are: (i) a science-based approach, relying on mass balancing, uncertainty assessment and review, and with focus on completeness, consistency and relevance for local decision support: (ii) a strong reliance on software-supported procedures using the latest advances in data science for data harvesting, uncertainty propagation, validation and review, and data presentation; (iii) a collaborative social interface, using open source and innovative social media technology for data

input, review, and interaction with the user communities. An underlying objective basic principle of the BONSAI network is the efficient use of information. This has two meanings: first, when a piece of information is generated there is no value added in regenerating it, unless this information is lost or not accessible. Thus, an efficient use of information should guarantee that, once generated, the knowledge contained in information is stored and accessible. Secondly, it means facilitating the generation of further value added by refining, expanding or connecting with other existing information. BONSAI is, therefore, not only about IO or process-based inventory data but also procedures such as algorithms and software and data for impact assessment that are needed to generate a sustainability footprint, and thus, also the resulting footprints itself. The present beta version of BONSAI includes primary features such as data download and upload and provision of a first report of the result per product or product comparison. The application and qualification of the feature-complete version of BONSAI, where *all the planned* features will be implemented and tested is the objective of a later Release Candidate version. Therefore, the beta version intends to be a proof-of-concept and an open platform for testing activities and bug fixing, in order to minimize the impact on users.

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Regional and temporal variation in environmental impacts from household consumption
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Environmentally extended input-output analysis (EEIOA) is often used to determine environmental impacts from household consumption, because it offers a complete and consistent framework. The analysis of regional (subnational) variation of household consumption is difficult, due to the low availability of regional IO models. However, such an assessment is particularly relevant when regional differences do occur: in Belgium, variation in population, income level and economic performance can be observed. This work builds on regional IO data to investigate to what extent this regional variation extends also to environmental impacts from household consumption. An environmentally extended multi-regional input-output model is used that shows a subnational resolution for Belgium (Brussels, Flanders and Wallonia). The regional household expenditure for 2003, 2007 and 2010 indicates the consumption of 81 products groups, seperated into Belgian products, European and non-European imports. The environmental extensions show 53 emissions and resources. By applying the algorithms of EEIOA, the direct and indirect emissions and resource use from household consumption are calculated and interpreted in terms of environmental impacts based on the ReCiPe impact assessment method. The development of environmental impacts from total household expenditure shows an increasing trend for each region over the three reference years. The development of impact intensities per habitant shows a regional variation (coefficient of variance) between 5 % and 11 %. Brussels impacts are the lowest in most impact categories. However, the existing variation, especially in the 2010 datasets is low. For most of the midpoint categories there is no systematically higher or lower enviornmental impact from household consumption between Flanders and Wallonia. The midpoint impacts from 2010 are further distinguished according to the different expenditure categories (such as food, clothing, housing, etc.) and direct impacts. The results show in general a relatively homogenous distribution over household expenditures in the three regions. The results indicate temporal and regional variation, but the variation in regional consumption seems less systematic than the temporal variation. An alignment in consumption level occurred over the three years. The small differences that were found represent specific urban conditions (housing and transport) that cannot be transferred on the rural regions.

The role of ecotoxicology in fisheries science and aquaculture (I)

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Controlling the effect of aquaculture on the environment
. Oaland, Marine Harvest ASA
We live in a world that is facing major environmental challenges, including climate change and the depletion of natural resources, but also a world where future food production needs to match global demand. Aquaculture can improve the worldâ_s standard of living by producing food that combines quality and healthy eating, while at the same time delivering a reduced carbon footprint. Of all farmed vertebrate production, fish farming is one of the most climate-friendly ways of producing protein. Marine Harvest is one of the largest seafood companies in the world and the world's largest supplier of farmed Atlantic salmon, providing 5.5 million salmon meals every day. We employ 12 717 people and are present in 24 countries. Marine Harvest integrates the entire value chain offering an opportunity to optimize our products from feed to fork, and be proactive in addressing challenges related to sustainable feed, farming and value-added processing. We aim to unlock the potential of the ocean by producing more food from the sea in a sustainable way, a vision we call Leading the Blue Revolution. Therefore producing in an environmentally and socially responsible way is a top priority. Our environmental and sustainability strategy focus on the following material aspects and ambitions: \n \n1. Climate friendly food production - we aim at reducing our GHG emissions by implementing energy saving initiatives in our operations; 2. Escape prevention – we have a target of zero escapes which we implement by a combination of several tools including risk assessment and operational standards, use of industry standards, global and local training, sharing of experience, use of more resilient nets and R&D efforts to enable tracing of escaped fish; 3. Sea lice management – based mainly on non-medicinal approaches and reduced medicine use; 4. Medicine use – focused on the implementation of non-medicinal methods and use of vaccines to limit dependency and use of antibiotics in bacterial disease control; 5. Biodiversity – in addition to monitoring our potential benthic impact, we also operate according to several standards that aim at preserving biodiversity including Global GAP, BAP and ASC and 6. Sustainable feed – we have developed a global sustainable feed policy which secures the use of sustainable raw materials. \n \nMarine Harvest's aim at being at the forefront in growing food from the ocean in a socially and environmentally responsible way is reflected in our commitment to become 100% certified against the strictest environmental and social standard available for farmed fish, the Aquaculture Stewardship council (ASC) standard. In 2016, Marine Harvest accounted for 38% of all ASC-certified farmed Atlantic salmon. Also integral to our vision of leading the blue revolution is our R&D efforts. We are currently evaluating different technology platforms which include systems for closed and semi-closed production systems, but also technology platforms allowing farming more off-shore thus limiting environmental impacts and allowing for a sustainable growth of the industry. \n

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The use of aquaculture medicines to control sea lice should not cost the earth
A. Lillicrap, NIVA Norwegian Institute for Water Research

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Emerging contaminants and toxins in aquaculture: how modern analytical tools may aid to ensure food and environmental safety
L. Vanhaecke, Ghent University / Veterinary Public Health and Food Safety - Laboratory of Chemical Analysis; S. Huysman, Ghent University; L. Van Meulebroek , Ghent University / Department of Veterinary Public Health and Food Safety
As a result of governmental incentives and technological developments, aquaculture has become a well-established and strongly growing segment in food production. To date, about 45% of worldwide fish supply is provided through aquaculture, but a continuously increasing trend may still be noted. In this regard, a deepened knowledge on adequate feed formulations, growth conditions, and disease management is of outmost importance. However, the intensified aquaculture process requires a high input of diverse resources, which may strongly impact the environment as well as food safety. Indeed, various chemical agents such as antibiotics, herbicides and algaecides are used, aiming at the promotion of the cultivated species by eliminating their major competitors. Although this strongly advances production, some adverse side-effects may be noted. For example, the administration of antibiotics, used to kill or inhibit the growth of bacteria, may also affect nontargeted species and induce antibiotic resistance. These antibiotics are generally present in the feed formulations, together with diverse other substances such as pharmaceuticals, anaesthetics, vitamins, and pigments, which may all accumulate in the environment. The same is true for herbicides and algaecides, frequently applied to control aquatic weeds, algal blooms and fouling organisms. As such, a variety of biological active and possibly toxic chemicals are introduced in the environment and may accumulate in edible species. Hereby, lipophilic emerging contaminants tend to bio-accumulate, whereas hydrophilic compounds are mostly converted to metabolites or derivatives, which may exert even more toxic effects than the parent compounds. In this context, the increasing prevalence of toxin producing species (algae blooms) is also assigned substantial significance as the produced biotoxins may strongly affect seafood growth rate, death rate and food safety. \nWithin this context, large-scale research is necessary to acquire valuable insights on 1) the presence of contaminants in our marine environment and aquaculture production systems, 2) the uptake, bioaccumulation and metabolisation of bioactive substances by (edible) species, and 3) the health risks associated with these substances and their derivatives. Hereby, new tools for monitoring (e.g. passive samplers) as well as high-end analytical instruments (e.g. high-resolution mass spectrometry for profiling and fingerprinting) are becoming indispensable to acquire a correct view on the impact and importance of contaminants. During this keynote, the urge of contaminant analysis and adequate monitoring will be demonstrated using practical examples. From this, current shortcomings and future needs for a better food safety warranting and sustainable production will be proposed.

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Contaminant control and development in farmed salmon- industry data
. Oaland, Marine Harvest ASA
Marine Harvest is one of the largest seafood companies in the world and the worldâ_s largest supplier of farmed Atlantic salmon globally. We control the full value chain and is an integrated company controlling breeding, feed, farming, processing and sales and marketing. The company employ 12 500 people and are present in 24 countries. \nWe aim to be an integrated provider of proteins from the

ocean, taking the lead in all key areas, from the production of fish feed to meeting the needs of the market. By integrating the entire value chain, we can control our products from feed to fork, and be more proactive in addressing challenges related to sustainable feed, farming and value-added processing. \nProviding healthy and safe seafood for a growing population require attention to both risks and benefits. With a significant change in salmon diet composition over the last decades this has also altered the picture with regards to contaminants. A typical salmon diet around year 2000 contained fish meal inclusion at 40-45% and fish oil inclusion around 25-30% (total 6575% marine raw materials) while todays feed composition is typically 10-15% fish meal and 10% fish oil (total 20-25% marine raw materials). \nData from Marine Harvest's global internal contaminant monitoring programme, run over the last 10-15 years, shows that the level of persistent organic pollutants has been reduced significantly. The reduction reflects the decreased inclusion level of meals and oils of marine origin, the main source of persistent organic pollutants in the salmon diets, and thus reduced contaminant level in the diets and the salmon fillets. \nOur data shows that the farmed salmon contaminant level for Dioxins and Dioxin-like PCBs are currently in the range of 6-8% of the statutory limit established in the EU. \n

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Sex steroids in common mussels: where do they come from and what risk do they pose to consumers?

I. Katsiadaki, Cefas / Environment and Animal Health; T.I. Schwarz, B.H. Maskrey, Cefas Weymouth Laboratory; A.P. Scott, Centre for Environment Fisheries and Aquaculture Science

The presence of the vertebrate sex steroids, testosterone (T) and 17β-estradiol (E_2) in mollusc tissues is often cited as evidence that they are involved in the control of reproduction in this phylum. We show here however, that the most likely source of T and E_2 in at least one species, the common mussel, *Mytilus spp.*, is by uptake from the water (i.e. the steroids are likely of an 'exogenous' as opposed to 'endogenous' origin). When mussels were exposed to radioactive T and E_2 in the water, levels fell rapidly and exponentially during the course of the exposures (up to 24 h). The 'clearance rate' over the first 1.5 h was calculated and this showed that a single mussel was able to clear the amount of radiolabel that was in c. 40 mL of water in one hour. The rate of uptake of radiolabel could not be saturated by the addition of up to 25μg/L non-radiolabelled T and E_2 in the water, indicating that the mussels have a very large capacity for vertebrate sex steroid uptake. Extraction and phase separation of radioactivity from the soft tissues of the animals showed that the bulk of the radiolabel (>70%) was present in the form of fatty acid esters. Only a small amount was present as free steroid. Importantly, we have discovered that T undergoes metabolism prior to fatty acid conjugation in mussels. Upon saponification of the esters followed by chromatographic separation, it was discovered that only c. 10% of the radioactivity remained as T itself. Instead, it was present in the two major steroids (each forming c. 40%) 5α-dihydrotestosterone (5α-DHT) and 5α-androstane-3β,17β-diol. E_2 is not transformed before esterification. We have also discovered that, as with rate of uptake, the rate of ester formation, for both steroids, could not be saturated. Two depuration experiments were carried out, both lasting ten days, and in neither was there a significant drop in the total amounts of radioactivity in the tissues. The only significant change was a drop in free steroid levels over the first 1 to 2 days. Our data provide compelling evidence that a) the origin of vertebrate steroids found in molluscs is not endogenous as widely accepted in the literature over several decades, b) the biotransformation of T and accumulation of two potent vertebrate receptor agonists in mussels may constitute a hazard for shellfish consumers that is currently not considered by regulators.

Toxicology and ecotoxicology: bridging the gaps (Part I: contemporary toxicological challenges)

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Harmonisation of Human and Ecological Risk Assessment of Combined Exposure to Multiple Chemicals: A Food Safety Perspective

J. Dorne, European Food Safety Authority EFSA / Scientific Committee and Emerging Risks Unit Department of Risk Assessment; C. Hogstrand, Kings College London / Division of Diabetes and Nutritional Sciences

Over the last few years, new developments towards harmonisation of methodologies for human and ecological risk assessment (HRA and ERA) of combined exposure to multiple chemicals ("chemical mixtures") have emerged particularly in the food safety area. Key challenges to harmonisation include the large number of chemicals involved, their associated exposure patterns, toxicological profiles in humans and species of veterinary and ecological importance as well as the diversity of regulations and associated RA frameworks. In principle, the purpose of the HRA or ERA is focused during the problem formulation phase and tiered approaches are applied to exposure assessment and hazard assessment combined for risk characterisation and uncertainty analysis. The application of these tiered approaches depends on the purpose of the HRA or the ERA, data availability, time and resources. These range from qualitative, semi-quantitative to fully probabilistic approaches. The purpose of this overview is to bring a historical perspective on HRA and ERA frameworks dealing with combined exposure to multiple chemicals and a brief account of the current work of EFSA's Scientific Committee to move towards harmonisation of these RA methodologies in food safety. In addition, new insights from data collection and research both in the human health and ecological area are illustrated to emphasise further opportunities for harmonisation. Particular emphasis is given here to weight of evidence approaches tailored to HRA and ERA. Specific examples are given for the integration of historical data and mechanistic alternatives to animal testing such as *in silico* and *in vitro* tools. The translation of research and the development of *in silico* models into open source platforms are discussed as the means for 1. providing scientific tools directed towards the RA and regulatory community 2. applying harmonised methodologies and 3. training the current and next generation of risk assessors.

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Ecological risk assessment of chemical mixtures

A.M. Ragas, Radbound University / Department of Environmental Science

Ecological risk assessment of chemicals has much in common with its human counterpart, but there are also distinct differences. One area where both fields can learn from each other is the assessment of chemical mixtures. A state-of-the-art overview of ecological risk assessment of chemical mixtures will be presented; a parallel presentation will focus on the human dimension of mixture assessment. Examples will be presented to illustrate the potential of individual-based modelling for assessing the time- and space-integrated exposure of ecological receptors to chemical mixtures, and for the propagation of effects on individual organisms to higher levels of biological organization, i.e., the population and ecosystem level. When it comes to the assessment of mixture effects, distinction is made between whole mixture approaches and component-based approaches. Whole mixture approaches, such as Whole Effluent Toxicity (WET) testing, are widely used in ecological risk assessment, mainly because toxicity tests with ecological receptors, particularly lower organisms, are less controversial than with human receptors. Component-based approaches are based on the widely used concepts of concentration addition and independent action (response addition), or combinations thereof. Approaches to deal with potentiation (synergism) or attenuation (antagonism) of toxicity are limited in the ecological arena. This may be explained by the limited mechanistic focus of ecological assessments. Refinement of ecological mixture assessments (e.g., as in tiering) focuses on improving ecological realism, e.g. by including ecological interactions, whereas refinement of human assessments focuses on improving the mechanistic understanding, particularly on the molecular level. The development of new techniques such as assay-based effect monitoring and passive sampling in combination with the determination of total molarity provide promising new opportunities to assess the ecological risks of chemical mixtures.

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Endocrine disruption: A toxicologist's perspective

S. Munn, European Commission

An endocrine disrupting substance according to the 2002 WHO/IPCS definition is an '*exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations*'. There is a raft of test guidelines developed and being developed within the context of the OECD for assessing a substance's potential to disrupt the endocrine system. Although phenotypic changes as an outcome of chemical toxicity across taxonomic groups may seem to vary considerably the initial interaction of the chemical at molecular and cellular level may be strikingly similar, particularly when considering receptor mediated effects for e.g nuclear hormone receptors where the receptor is considered well conserved across different taxa. For this reason, *in vitro* screening assays within the OECD Conceptual Framework for the testing and assessment of endocrine disrupting substances, although based on mammalian cells, are used as an indication of endocrine activity relevant for an assessment of endocrine disruption potential for human health and ecotoxicological assessments. Although toxicokinetic differences between taxa and species may have a big impact on toxicity, use of adverse outcome pathways to elucidate the similarities and differences between different taxonomic groups can be explored to help assess the applicability of different types of assays for different purposes. The presentation will attempt to focus on ways of developing a more holistic approach to identification of EDCs for both wildlife and humans.

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Endocrine disruption: An ecotoxicologist's perspective

J. Wheeler, Dow Agrosciences

Endocrine disrupting properties require specific evaluation under several legislations globally. The development of specific criteria to "identify endocrine disrupting properties" is underway to enable hazard-based regulation in the European Union. Whilst in the United States and Japan, scientific, risk-based approaches are being developed. Both approaches require assessments for human health and the environment (wildlife). Regardless of the regulatory process, most geographies use the World Health Organisation International Programme on Chemical Safety definition of an endocrine disrupter or variants thereof. This requires the identification of an adverse effect, underlying endocrine mechanism and establishing a plausible link between the two. The environmental and human health approaches differ according to their respective protection goals. Either at the individual or population level. Ecotoxicologists accept some individual level effects as long as population stability and recruitment are not impacted. Such determinations rely on biological, statistical, design and potentially ecological modelling for sound interpretation. This can often mean the same data may be interpreted very differently for the protection of individual human health compared to wild populations (e.g. wild mammals). It has also led to the development of many new screening and definitive tests in multiple taxa. These traditionally are outside of the focus of human health being in non-mammalian models (fish, amphibians and birds). However, for endocrine disruption they have been shown to be good general models for specific activities (e.g. amphibian metamorphosis and thyroid activity) or particularly sensitive indicators in screening batteries (e.g. fish for steroidogenesis). Ecotoxicology has historically focused on adverse effects of concern (growth, development and reproduction) and less so on a mechanistic understanding. Consequently, the regulation of endocrine disruption based partly on establishing mechanisms requires a better understanding more in line with toxicological approaches. Considering the need to screen so many substances, the drive to higher throughput approaches and the conserved nature of the vertebrate endocrine system the need for collaboration between ecotoxicology and toxicology has never been greater.

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Panel discussion

Combined effects of chemical and environmental stressors: from local stressors towards climate change (III)

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Influence of temperature and moisture on toxicity of propiconazole and chlorantraniliprole to earthworm Eisenia fetida
B.K. Hackenberger, G. Palijan, . Lonńarī, Department of Biology University of Osijek; D. Hackenberger, Department f Biology, University of Osijek
Earthworms became the most used biological model in toxicity assays and environmental monitoring studies, particularly for the toxicity of pesticides on soil ecosystems. Although some pesticides are reported safe for non-target invertebrates there are not tested with possibility of soil temperature and moisture regime change. Results of many studies show a general warming in all seasons and soil depths in the observed periods for soil temperatures, with change in soil moisture as well. The aim of this study is to investigate the biochemical responses of earthworm Eisenia fetida and its gut microbial activity after exposure to propiconazole (PCZ) and chlorantraniliprole (CAP) under different temperature and moisture regimes. Chosen temperatures were 20 C and 25 C and soil moisture 30% and 50%. Activities of acetylcholinesterase (AChE), catalase (CAT) and glutathione-S-transferase (GST) were measured after 7 days of exposure and, after transfer to clean soil, 1, 3, 5 and 10 days post exposure. Additionally, gut microbial activity was investigated by conducting crystal violet (CV) test and dehydrogenase activity (INT) measuring on earthworm faeces obtain after exposure and each post-exposure sampling. Some of the results were as expected considering the stress elicited by higher temperature and low soil moisture, but the increased activity of acetylcholinesterase obtained after exposure was not expected.

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Chlorpyrifos-induced oxidative damage is reduced under warming and predation risk
L. Janssens, KULeuven; R. Stoks, University of Leuven / Department of Biology
Organisms are confronted with a variety of anthropogenic and natural stressors that can interact with pollutants. Identifying and understanding why and when such interactions occur is a major challenge for ecological risk assessment. Especially at the physiological level interactions between stressors are poorly studied, despite their importance to understand and explain interactions at higher levels of organization. We investigated the single and combined impact of the pesticide chlorpyrifos, warming and predation risk on traits related to oxidative stress using damselfly larvae as study organisms. To understand the underlying mechanisms we integrated information on two important reactive oxygen species (ROS) (the superoxide anion and hydrogen peroxide), two key antioxidant enzymes in insects (superoxide dismutase [SOD] and catalase [CAT]) and oxidative damage to lipids (levels of malondialdehyde [MDA]). Chlorpyrifos exposure resulted in increased ROS levels, decreased antioxidant defence and increased oxidative damage. The larvae coped better with the pesticide when reared at the higher temperature as a result of a higher investment in antioxidant defence. Although also predation risk increased oxidative damage, the pesticide effects on oxidative damage were less strong in the presence of predator cues (at 20°C). Despite the weaker pesticide-induced effects under predation risk, the combined exposure to the pesticide and predator cues consistently resulted in the highest ROS levels, the lowest antioxidant defence and the highest oxidative damage. Our results provide the first evidence for antagonistic interactions with a pollutant for physiological traits linked with oxidative stress.

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Strong differences between two congeneric species in sensitivity to pesticides in a warming world
L. Op de Beeck, Laboratory of Aquatic Ecology & Evolutionary Biology - KU Leuven / Biology; J. Verheyen, University of Leuven / Laboratory for Aquatich Ecology, Evolution and Conservation; R. Stoks, University of Leuven / Laboratory of Aquatic Ecology Evolution and Conservation
Freshwater biodiversity is particularly vulnerable to global warming and pesticides. Both stressors are directly linked since most pesticides become more toxic at high temperatures (climate-induced toxicant sensitivity, “CITS”). This may, however, not reflect the net effect of pesticides in nature when their degradation is more rapidly at higher temperatures. Furthermore, exposure to pesticides can reduce the tolerance to extreme temperatures (toxicant-induced climate sensitivity, “TICS”). Understanding how both stressors interact is therefore crucial to correctly estimate the impact of pesticides in a warming world. We compared the effects of the pesticide chlorpyrifos (CPF) under warming on life history traits, thermal tolerance (CTmax) and physiology of larvae of two *Ischnura* damselfly species. Based on energy-allocation theory and empirical across-species trait-based sensitivity patterns for CPF, we expect the much faster life history, the associated higher metabolic rate, and the smaller size of *I. pumilio* to result in a higher sensitivity to CPF compared to *I. elegans* and therefore also a higher “TICS”. CPF negatively affected survival and growth rate, but these effects were much smaller in *I. pumilio* than *I. elegans*, indicating a much lower sensitivity. This difference in sensitivity could be explained by a higher fat content, and higher activities of acetylcholinesterase and of detoxifying and anti-oxidant enzymes. While for *I. pumilio* the effect of CPF was small and did not depend on temperature, for *I. elegans* the impact of CPF was higher at 20°C compared to at 24°C, which could be explained by the higher accumulation of the pesticide after multiple pulses at 20°C than at 24°C. As a result of its low sensitivity, CPF did not reduce the CTmax of *I. pumilio* while it did for *I. elegans*. Our results demonstrate that closely related species can have very different sensitivities to a pesticide resulting in species-specific support for “TICS” and “CITS”. The fast growing, small *I. pumilio* was 8x less sensitive to CPF than the slow growing, large *I. elegans*. This contradicts expectations based on energy allocation trade-offs and empirical across-species trait-based sensitivity patterns for CPF, and indicates that interspecies extrapolation of sensitivity estimates to pesticides based on taxonomy and on currently used traits can be misleading, even for closely related species.

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Sequential pesticide exposure increases toxicological sensitivity of crustaceans
R. Russo, Helmholtz centre for environmental research - UFZ / System Ecotoxicology; J. Becker, M. Liess, UFZ Center for Environmental Research / Department of System Ecotoxicology
The widespread existence of pesticide-related impacts on ecosystems suggests that the current approach of pesticide risk assessment fails to protect biodiversity. One issue is that regulatory decisions are based on tests with a single exposure, while agricultural ecosystems are generally threatened by the successive contamination of pesticides. Here, we investigated whether individuals of the crustacean *Gammarus pulex* exposed to pesticides in agricultural streams develop resistance or suffer a higher sensitivity following sequential insecticide exposure. We revealed that individuals from contaminated sites showed 2.7 fold higher sensitivity to the insecticide esfenvalerate in acute laboratory tests compared to individuals from reference sites. Additionally, we revealed that within the range of our data, temperature stress further increased the pre-exposure weakening of crustaceans by a factor of 2. Our findings show that sequential exposure and moderate environmental stress may increase individual sensitivity to pesticides. We conclude that future risk assessment models either need to consider these processes or the latter should be reflected within the applied safety factors.

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Combined effects of water scarcity and an insecticide on freshwater zooplankton communities: a microcosm study
A.A. Sanchez, IMDEA Water Institute / Aquatic Ecotoxicology; I. López, IMDEA Water Institute; A. Rico, IMDEA Water Institute / Aquatic Ecotoxicology; M. Vighi, IMDEA Water Institute / Earth and Environmental Sciences
Water scarcity is an expanding climate and human related condition, which drives and interacts with other stressors in freshwater ecosystems, such as chemical pollution. The increasing global pressure on water resources, particularly in arid and semi-arid areas, indicates that the interaction between these two groups of stressors is going to increase in the nearby future. In this study we evaluated the combined effects of the insecticide lufenuron and two stress factors: increasing water temperatures and droughts. Twenty-seven microcosms were stocked with pond water, sediment, and a homogeneous plankton assemblage. Two different treatments corresponding to two different temperatures (20°C and 28°C), and another treatment characterized by the highest temperature and desiccation as additional stress factor were evaluated. The experiment was performed in triplicate with three insecticide treatments (Control, Low and High Concentration) in each temperature-desiccation treatment. The insecticide was applied twice, on day 0 and on day 10 after the start of the application period. In the test units in which there was

no desiccation, microcosms were refilled with distilled water simulating rainfall contributions. In the test units exposed to desiccation stress water was not refilled and were allowed to dry (and were refilled again after desiccation). The concentration of lufenuron in water and sediments were periodically measured, together with water quality parameters (DO, pH, T, electric conductivity, nutrients, chlorophyll, etc.). Zooplankton composition was checked weekly by microscopic counting, and the interactive effects of temperature, drought and lufenuron were evaluated using suitable statistic techniques. Lufenuron applications resulted in a significant decrease of large zooplankton, primarily cladocera, and on an increase of rotifera. From the evaluation of the zooplankton dynamics on day 14, it can be concluded that temperature had no major effects on the community composition, but influenced their vulnerability to the insecticide stressor. However, insecticide effects were not found to be larger in the desiccation treatment as compared to the non-desiccation treatment. The evaluation of the combined stressor long-term effects and population/community recovery is still undergoing. The results of this study contribute to describe the differential vulnerability of aquatic ecosystems to multiple stress factors in arid and less arid regions.

Polar ecotoxicology: hot issues in cold climates

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Effects and uptake and excretion kinetics of 11 selected PAHs in Arctic lipid rich copepods

I. Salaberria, NTNU University / Biology; I. Øverjordet, SINTEF Materials and Chemistry; R. Nepstad, SINTEF Materials and Chemistry / Monitoring and Modelling; B. Hansen, SINTEF Materials and Chemistry; T. Nielsen, DTU Aqua / Technical University of Denmark; D. Altin, BioTrix; T. Nordtug, SINTEF Materials & Chemistry / Environmental Technology; A. Olsen, Norwegian University of Science and Technology

Increased ice cap melting is opening up new areas in the Arctic to human activities such as oil exploration. Offshore oil production releases large quantities of hydrocarbons into the marine environment, notably as produced water and oil droplets. The PWC-Arctic project endeavors to assess the potential environmental impact of oil exploration in the High North by quantifying the uptake and excretion kinetics of selected produced water components and oil droplets and their effects on growth, development and reproduction of the Arctic copepods *Calanus glacialis* and *C. hyperboreus*. These calanoids are key zooplankton species with high lipid content, which makes them prone to uptake and accumulation of lipophilic oil components. Particular focus is on bioaccumulation kinetics, oil component transfer, effects on respiration, growth, fertility and fecundity. Experimental findings suggest that the physiological effects of short term exposure to environmentally relevant concentrations of 11 selected polyaromatic hydrocarbons (PAHs) are minor. However, several of the heavier PAHs such as fluoranthene, pyrene and chrysene showed up to approximately 100 % retention after a 120 h depuration period. This slow depuration of PAHs in lipid rich Arctic copepods indicates that stored produced water components may be more available for transfer to progeny and to higher trophic levels than previously anticipated.

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Contaminants and energy expenditure in an arctic seabird: Organochlorine pesticides and perfluoroalkyl substances are associated with basal metabolic rate in a contrasted manner

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Basal metabolic rate (BMR), the minimal energetic cost of living in endotherms, is known to be influenced by thyroid hormones (THs) which are known to stimulate in vitro oxygen consumption of tissues in mammals and birds. Several environmental contaminants may act on energy expenditure through their thyroid hormones-disrupting properties. However, the effect of contaminants on BMR is still poorly known for wildlife. Here, we investigated the relationships between three families of contaminants (organochlorines, perfluoroalkyl substances (PFASs), and mercury) with BMR and circulating total THs (thyroxine (TT4) and triiodothyronine (TT3)) in arctic breeding adult black-legged kittiwakes (*Rissa tridactyla*). Our results indicate a negative relationship between chlordane mixture (∑CHLs) and BMR in both sexes whereas perfluorotridecanoate (PFTrA) and BMR were positively related in females only. BMR was not associated with mercury burden. Additionally, levels of TT3 were negatively related to ∑CHLs but not to PFTrA. Organochlorines and PFASs may therefore disrupt fine adjustment of BMR during reproduction in adult kittiwakes and this may cause fitness penalties. Importantly, highly lipophilic organochlorines and highly proteinophilic PFASs appear, at least in females, to have the ability to impair self-maintenance in an opposite way. Therefore, our study highlights the need for ecotoxicological studies to include a large variety of contaminants which can act in an antagonistic manner.

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Combined effect of sea ice retreat and pollutants on lipid metabolism in polar bears

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In the current context of climate change, a phenological mismatch between predators and their prey distribution is expected for a number of species. In the Arctic, the amount of sea ice is steadily decreasing. In response to seasonal prey availability and cold temperature, Arctic marine mammals have evolved large body fat stores which they accumulate during periods of prey availability and mobilize when food is scarce. Polar bears (*Ursus maritimus*) accumulate large fat stores during the ringed seal availability peak (spring to early summer). In contrast, during the late summer-autumn period, food encounters are in many areas rare and a large proportion of polar bears enter a fasting state. In addition to the threat of ongoing global warming, polar bears are exposed to a number of lipophilic and proteinophilic pollutants (legacy POPs, brominated flame retardants, PCB metabolites and PFASs) which are related to a number of health effects. Although adipose tissue functions to store energy, it is also a major endocrine organ. The relationships between environmental pollutants and lipid metabolism have received increasing attention over the last decades but still need to be adressed in wildlife. We tested whether the combined effects of sea-ice conditions and environmental pollutants could synergistically affect lipid metabolism in female polar bears. We investigated the relationships between pollutants and markers of lipid metabolism at different physiological levels (transcriptomics, fatty acid composition, clinical chemical parameters) in Svalbard female polar bears sampled during two contrasted seasons (spring, when sea-ice is extended and autumn when most sea-ice has retreat), years (2012, more sea-ice in winter than in 2013) or habitats (Western Svalbard has less sea ice year long than Eastern Svalbard). We show that lipophilic pollutants increased the expression of genes involved in lipid metabolism. For example PFAS concentrations increased the expression of a gene indirectly required for cholesterol synthesis, modified the composition of saturated and monounsaturated fatty acids in adipose tissue and increased the concentrations of lipid associated parameters in plasma. The results of this project all agree to describe a disruption of lipid metabolism in female polar bears from Svalbard and that polar bears using a less predictable environment would be more at risk than individuals using a predictable environment, regardless the habitat quality.

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Are snow buntings (Plectrophenax nivalis) in Svalbard affected by local urban pollution?

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Local urban pollution issues are of increasing concern in the Arctic. Longyearbyen is the largest settlement in Svalbard, Norway, and Longyearbyen, including Adventdalen is an area with an extensive history of coal mining. In additon, emissions of potentially toxic elements to the local environment may occur from the coal-fired power plant in Longyearbyen, and urban related activities in this town. In the present study, the snow bunting (*Plectrophenax nivalis*), which is the northernmost nesting passerine species and the only passerine that regularly breed in the high Arctic environment of Svalbard was applied as an indicator species to study accumulation of urban derived pollution in the Longyearbyen and Adventdalen area. The main objective was to assess how accumulation of elements varies with sex and age in snow buntings, and to which extent the concentrations are associated with potential local pollution originating from mining activity, emmissions from the coal-fired power plant and other urban activities in Longyearbyen, Svalbard. Thus, feathers were sampled from adult-nestlig pairs of snow buntings. Significant differences in feather concentrations of elements were identified between adult and nestling snow buntings in Longyearbyen and Adventdalen. There were no sex-difference in the feather-concentrations in the adults birds. The concentration pattern of geogenic origining rare earth elements (REE), Mn and Cr in adult bird feathers indicates the possibility that external contamination of the feathers may 'mask' the actual internal burdens of elements. The results indicate elevated concentrations of some elements associated with urban activity. The higher concentrations of Hg in the nestlings as compared to the adults may be of concern. *Acknowledgement* – The study was funded by the VINTERSPURV project 'Reproductive biology of the snow bunting (*Plectrophenax nivalis*)' funded by the Svalbard Evnrionmental Protection Fund.

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Causes and consequences of mercury exposure in an Antarctic seabird

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Mercury (Hg) is a pervasive contaminant reaching remote environments through atmospheric transport and deposition. Seabirds as predatory species accumulate high quantities of Hg through food intake. Reproduction has been identified as one of the most sensitive endpoints of Hg toxicity in marine birds. Yet, few studies have attempted to quantify the causes and consequences of Hg exposure in Antarctic seabirds, where increasing environmental perturbations challenge animal populations. Here we focus on a large sample (N = 266) of Antarctic petrels *Thalassoica antarctica* from Svarthamaren, Antarctica. Hg and the stable isotopes of carbon ($\delta^{13}C$, proxy of feeding habitat) and nitrogen ($\delta^{15}N$, trophic position) were measured in whole blood, and breeding behaviour monitored over two seasons (2012-13, 2013-14). Our aims were to 1) quantify the influence of individual feeding ecology ($\delta^{13}C$, $\delta^{15}N$ and GPS tracking) on Hg exposure and 2) test the relationship between blood Hg concentrations and breeding output. Blood Hg concentrations in Antarctic petrels ($0.86 \pm 0.24\ \mu g.g^{-1}$ dw in 2012-13) are relatively low when compared to other species of Antarctic seabirds. Blood Hg concentrations were positively and significantly related to blood $\delta^{15}N$ values, indicating Hg biomagnification in Antarctic food webs. By contrast, there was no evident effect of feeding habitat (inferred from both GPS tracks and $\delta^{13}C$ values) on Hg exposure, suggesting that Hg contamination in Antarctic food webs is relatively homogeneous over a large geographical scale. We document no relationship between blood Hg concentrations during the incubation period and short-term breeding output (hatching success and chick survival at 15 days). Nevertheless, further studies on pre-laying Hg exposure and long-term breeding output are warranted in this population. This is pivotal in the context of rapid ongoing environmental change that strongly inflences Svarthamaren Antarctic petrel population dynamics and which can modify Hg distribution patterns in polar environments.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (III)

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REEchangE-Rare Earth Elements in a Changing Environment

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REEchangE focuses on the anthropogenic release of the rare earth elements (REE) lanthanum and gadolinium to the environment and their potential risk within ecosystems. REE are increasingly applied e.g. in green technology, and consequently also emitted to the environment. But there is a diversity of potential polluting sources of which little is known, and no regulatory environmental framework exists so far. Considering their future use, release, and environmental fate, an evaluation of environmental risk from Lanthanum and Gadolinium will have to be based on information on exposure pathways, and exposure and effect concentrations. In a current review by Herrmann et al. (2016) the considerable lack of reliable data for REE toxicity in sediments and in the marine environment has become apparent. The project REEchangE focuses on the aquatic environment and here tackles those aspects in two ways: (1) By substance flow analysis (SFA), exemplarily performed for Germany. Information has been collected from published work for a variety of potential sources for La and Gd in rivers and lakes. Additionally, water and sediment samples have been analysed at specific locations. Current data point to wastewater and specialised industries as prominent sources of emission. (2) By investigating the impact of changing environmental parameters (pH, redox, salinity) on the bioavailability of particle bound La and Gd. In a microcosm, a battery of miniaturized biotests will be applied to monitor the toxicity responses in overlying water and sediment, also after simulated resuspension events. These experiments are currently ongoing. Results on ecotoxic responses obtained for *Aliivibrio fisheri* and *Rhaphidocelis subcapitata* so far are in the same range as literature data, and show a higher toxicity of Gd compared to La. Compared to common heavy metals toxicity also appears to be in a comparable range of concentration. **Organism EC_{50}Gd EC_{50}La** *A. fischeri* (this study) 23.45 μg/ml 71.76 μg/ml *P. subcapitata* (this study) 0.945 μg/ml 11.32 μg/ml Keywords: Rare Earth Elements, Substance Flow Analysis, Aquatic Ecotoxicology, HERRMANN, H., NOLDE, J., BERGER, S. & HEISE, S. 2016. Aquatic ecotoxicity of lanthanum – A review and an attempt to derive water and sediment quality criteria. *Ecotoxicology and Environmental Safety,* 124**,** 213-238.

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Derivation of soil threshold concentrations for arsenic: evaluation of ecotoxicological data for several species in six German soils

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The German Federal Soil Protection Act (1988) defines precautionary values for seven metals which, if exceeded, indicate that concern for a harmful soil change exists, taking geogenic or wide-spread, settlement-related pollutant concentrations into account. All precautionary values given in the German soil protection Ordinance (1999) are based on total concentrations ("aqua regia"). Since total metal concentrations do not represent the actual exposure of soil organisms to these metals, a realistic risk assessment of metals should consider the bioavailability of metals in soil, e.g. by taking into account soil properties such as texture, pH, or organic matter content etc. or using other metal extractions, such as 0.01M $CaCl_2$, 1M NH_4NO_3, DTPA or weak acid, as the basis of the assessment. The aim of this study is to connect bioavailable fractions of arsenic with ecotoxicological effect concentrations. This metal was chosen since it had not been studied much so far. Six soils covering a wide range of Central European soil properties were chosen and spiked with sodium arsenate dibasic heptahydrate. In these soils microbes, plants and invertebrates were tested, according to the ISO standard guidelines. Test results (given as EC_{50} values based on aqua-regia extractable As concentrations) are compared with different soil properties (pH, organic carbon content, Al-, Fe-, and Mn-oxides, etc.) to indicate their influence on bioavailability and toxicity of arsenic in the chosen soils. So far a consistency of sensitivity values was observed among all test systems for the same soils. Particularly results for soils, which are low in organic carbon, exhibit lowest EC_{50} values. Soils with the lowest sensitivity outcome have high amounts of FE-oxides in common. Further aspects which will be evaluated and discussed are the suitability of the different soils to be used in ecotoxicological tests and suitability of the extraction method. In addition, our results may help in the future to identify soils which are "exceptional" in their properties – and whether such soils should be categorically included in metal risk assessment.

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Improved comparative toxicity potentials of 23 metallic elements in soils: addressing solid- and liquid-phase speciation in environmental fate, exposure, and effects

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Development of comparative toxicity potentials (CTP) of metals in soils for applications in hazard ranking and toxic impact assessments should consider speciation. The solid-phase speciation determines size of the metal pool in the soil that is potentially accessible for leaching and uptake by biota. The liquid-phase speciation determines the size of metal pool that is present in directly bioavailable, toxic metal forms. Here, we calculated CTPs of 23 metallic elements in soils, addressing solid- and the liquid-phase speciation, where relevant and where possible. CTPs were developed for: 1 alkali metal (Cs); 3 alkaline earth metals (Be, Sr, Ba); 12 transition metals (V, Cr, Mo, Mn, Fe, Co, Ni, Cu, Ag, Zn, Cd, Hg); 4 post-transition metals (Al, Tl, Sn, Pb); 2 metalloids (As, Sb); and 1 non-metal (Se), which is sometimes classified as a metalloid. CTPs were calculated for a set of 436 soils from around the World (ISRIC-WISE3 database) using USEtox, WHAM7 and empirical regression models. Terrestrial toxicity effect factors were either calculated using terrestrial biotic ligand models (for Cu and Ni), or were retrieved from freshwater ecotoxicity EFs assuming that aquatic species are equally sensitive to metal (free ion) exposure compared to terrestrial species. The largest median CTP values were calculated for Ag(I) and Al(III). The lowest median CTP values were calculated for Mo(VI) and Sb(III). The largest variability in the CTPs, by up to 6 orders of magnitude, was observed for elements which precipitate as hydroxides, namely Al(III), Cr(III) and Fe(III). The variability in the CTP was somewhat smaller for other elements (from 1 to 4 orders for magnitude). When compared with CTPs calculated with USEtox disregarding speciation (i.e. using USEtox-default distribution coefficients combined with total-dissolved based effect factors), our median CTPs were 0.5-4 orders of magnitude smaller for 9 substances, within a factor of three for 17 substances, and 1 order of magnitude higher for Ag(I)). Our median CTPs were generally higher by ca. 1 order of magnitude than the ReCiPe 2008 CTPs (with some exceptions), and were generally smaller by ca. 1-2 orders of magnitude than Impact 2002+ CTPs (again, with some exceptions). Our new CTPs including solid- and liquid-phase speciation can substantially deviate from model results that do not include speciation, suggesting that speciation is important for characterization of terrestrial ecotoxicity of metal emissions.

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The effect of ageing on lead toxicity to the soil invertebrate Enchytraeus crypticus

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The interaction of metals with soil is a dynamic process, which may also depend on the form in which the metal ends up in the soil. For a well-soluble metal form (e.g., $Pb(NO_3)_2$) sorption may increase with time, while for a poorly soluble form (e.g., PbO), dissolution may lead to an increase in availability with time. Therefore, it is important to assess the effects of these dynamics on metal bioavailability. The present study aimed at investigating the effect of ageing on the bioavailability and toxicity of lead nitrate ($Pb(NO_3)_2$) and lead oxide (PbO) in the potworm *Enchytraeus crypticus* in Lufa 2.2 natural soil. Worms were exposed to Pb shortly after spiking the soil and after ageing for 3, 6, 12 and 18 months. Survival and reproduction after 21 d exposure were related to total, 0.01 M $CaCl_2$ extractable and porewater Pb concentrations in the soil and internal Pb concentrations in the surviving animals. Here results are summarized for the first 6 months. Pb concentration in pore water showed a slight increase after 6 months for $Pb(NO_3)_2$-amended soils and increased more strongly for PbO-amended soils. The ionic strength of the pore water increased and pH decreased with ageing time for $Pb(NO_3)_2$-spiked soils, but only slighly for PbO. LC50 and EC50 values for the effect on enchtraeid survival and reproduction on the basis of total Pb concentrations in the soil did not differ for $Pb(NO_3)_2$ after 6 months, but decreased from 4803 and 151 to 2229 and 124 mg Pb/kg dry soil for PbO. LC50 based on 0.01 M $CaCl_2$ extractable and porewater Pb concentrations increased from 2.2 to 2.5 mg Pb/kg dry soil and from 0.25 to 0.33 mg Pb/L for $Pb(NO_3)_2$ but decreased from 3.0 to 2.4 mg Pb/kg dry soil and from 0.26 to 0.20 mg Pb/L for PbO after 6 months. LC50 and EC50 values related to internal Pb concentrations did not differ for both $Pb(NO_3)_2$ and PbO and were similar at t=0 and after 3 and 6 months. In general, survival of *E. crypticus* was better expained from internal Pb concentrations in the worms than from total or available Pb concentrations in the soil. When focusing on laboratory toxicity studies, the effect of ageing and chemicals selected should be taken into account.

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Laterite associated waters - What the lack of major ions means to trace element bioavailability and toxicity
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Currently, aquatic toxicity data for metals (and other contaminants) is more abundant for temperate than for tropical species. However, numerous comparative analyses have generally concluded that tropical and temperate species are similar in sensitivity to metals. In this paper, we postulate that the question of differential metal sensitivity between temperate and tropical species has not been asked within the proper framework. Specific to metals and other toxicants that disrupt ionoregulation, we hypothesize that the unusual water chemistry of waters associated with laterite geology has led to the evolution of aquatic organisms that ionoregulate differently than non-laterite species and these differences in ionoregulation may impart differences in metal sensitivity. Laterites and the related bauxites are an abundant class of soils and sub-soils in the tropics[1]. They are formed by extended weathering under tropical climatic conditions[2] resulting in a leached profile enriched in some elements but depleted in those that are soluble under the specific conditions of formation. The majority of research into trace metal bioavailability and toxicity in natural waters has been undertaken on temperate or tropical species from non-lateritic geology. However, as mining and associated activities increasingly develop a more tropical focus, and exploitation of lateritic and laterised deposits becomes a more common source of base metals globally, issues related to natural aquatic ecosystem composition and sensitivity to altered metal concentrations have increased focus on the nature of fresh waters associated with these regions. This paper examines some important characteristics of laterite-associated waters, their biodiversity, their ion chemistry and how that relates to potential trace metal bioavailability and toxicity.

Modelling and monitoring of pesticides fate and exposure in a regulatory context (II)

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Probabilistic approach for a countrywide risk assessment of watercourses exposed to spray drift in fruit growing in the Netherlands
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Deposits of spray drift onto surface waters contribute significantly to the risk of exposure to pesticides for aquatic organisms. This risk is particularly high for surface waters alongside pome fruit orchards, where pesticide sprays are applied in a sideways or an upward direction. Recently a spray drift model has been developed to estimate pesticide deposits onto downwind off-target areas next to fruit orchards. Using this spray drift model, an exposure assessment model has been developed to estimate risk of exposure to pesticides for aquatic organisms in edge-of-field watercourses next to pome fruit orchards in the Netherlands. This paper describes the probabilistic processes concerning the countrywide risk assessment using the exposure model. Spatial and temporal variables are distinguished. Spatial variables include regional distributions of orchards and different types of watercourses, various water levels and continuously varying growth stages during the year. Temporal variables include frequency distributions of wind speed and direction and ambient temperature. 90% risk levels of predicted environmental concentrations (PEC) in surface water can be determined for various spray application schemes including multiple spray applications during a year. In an extensive simulation study the PECs in the watercourses were computed for all possible spatial configurations. A spatio-temporal statistical analysis on these simulations resulted in a quantitative risk assessment for a representative set of spray application schemes. The model is not limited to the spray application scenarios that were studied. A wide range of spray application scenarios can be simulated, which include the potential use of spray drift mitigation techniques and crop-free buffer zones. All of these features result in a versatile exposure assessment model with a high level of realism. The spray drift deposits onto the water surface can be used as input for models describing the fate of pesticides in the watercourses. Currently, the present model is combined with the TOXSWA fate model. In this way, a realistic simulation study on the exposure to and fate of pesticides in surface waters can be performed to quantify exposure risk levels for aquatic organisms. This serves higher-tier assessment studies for the authorization of plant protection products.

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Options for the spatial statistical population of the exposure assessment goal for aquatic organisms at EU level
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Assessment of risks for aquatic organisms is an important aspect of the environmental risk assessment of pesticides in western countries. This risk assessment consists of a combination of an effect assessment and an exposure assessment. The aquatic effect assessment developed by EFSA is based on well-defined effect protection goals (called ‘specific protection goals’, abbreviated SPGs). Also the exposure assessment requires well-defined goals (abbreviated EAGs). The overall level of protection for aquatic organisms is thus the combination of the SPGs and EAGs. However, so far no EAGs have been defined for the EU aquatic risk assessment. Therefore this work deals with the definition of these EAGs. An EAG can be defined by specifying six elements including the spatial unit and the spatial statistical population of the spatial units (further abbreviated to SSPSU). The definition of the SSPSU is still controversial so this work focusses on this. The first step for the definition of the SSPSU is the definition of the total area. The best approach seems to choose a climatic zone within the EU in line with the current FOCUS groundwater and surface water scenarios. The next step is whether the SSPSU should be based on ditches/streams at the edge of treated fields or whether all ditches/streams in the landscape should be considered. The EFSA aquatic guidance refers to water bodies at the edge of treated fields so this is the obvious choice. The last aspect for the edge-of-field water bodies is whether their definition should be based on restrictions with respect to exposure routes. This restriction was included in the FOCUS surface water scenarios because these are combinations of either only runoff plus spray drift or only drainage plus spray drift, where spray drift is based on wind blowing perpendicular to the edge of field during application. There are two arguments against this restriction. The first is that there is no relation with the effect protection goal. The second argument is that the size of the SSPSU would decrease strongly for repeated applications because the probability of a wind angle perpendicular to the edge of the treated field is only 30/360 = 0.08 (assuming a margin of this angle to the perpendicular direction of 15^o). It is difficult to envisage a scientific rationale such an approach. Therefore it is recommended to define the SSPSU as all water bodies at the edge of treated fields in a climatic zone within the EU.

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SETAC DRAW: Modelling pesticide spray drift for regulatory risk assessment
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Pesticide spray drift, which is the movement of pesticide through the air as particles (usually in water droplets) is an important component of the risk for a wide range of non-target species, including humans, other mammals, birds, arthropods, plants and aquatic organisms. The main basis of spray drift exposure assessments in the EU is experimental data. However, spray drift models are available at member state level, and are used outside the EU and there has been a move towards the use of data generated by a drift model for determining bystander and resident exposures at EU level. This paper explores the benefits of modelling approaches for spray drift exposure assessments, reviews the models that are available and the extent to which they are currently used in regulation. The steps needed to obtain a model appropriate for predicting exposures to spray drift are outlined and a way forward, developed through the DRAW workshops, is proposed. It is concluded that pesticide spray drift models are already available and could contribute to significant advances in exposure assessments for a wide range of non-target species. While there is some use of spray drift models in pesticide regulation world-wide, this is very limited at EU level, leading to very large inconsistencies in the way exposures to spray drift are determined for different species. SETAC DRAW aims to find a consistent, realistic and harmonised approach to assessing spray drift exposures, and the use of models will be an important component of this.

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Comparison of aquatic exposure assessment models for pesticide use on rice
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An evaluation of six modeling approaches for predicting environmental concentrations associated with the use of crop protection chemicals on rice was conducted. A comparison of predicted environmental concentrations (PECs) computed with all six approaches (EPA Tier 1, MED-RICE, Japanese regulatory spreadsheet “Aquatic PEC”, SWAGW, RICEWQ-EXAMS, and PFAM) is presented along with the status of their regulatory acceptance for pesticide

registration in the United States, European Union, China, and Japan. RICEWQ-EXAMS is used in the Pesticide Risk Assessment Exposure Simulation Shell (PRAESS) for China Scenarios and for higher tier in Europe. Of the six models, RICEWQ and PFAM models have the capability to simulate multiple pesticide applications, metabolites, and the flooding, overflow, and controlled releases of water associated with rice production. Each country has different guidance on which model, input parameters and ecological environments are used for computing PECs. A description of each model/scenario will be presented a long with a comparison of PECs from two pesticides using each model.

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Mitigating pesticide runoff in an agricultural catchment

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Pesticide concentrations in rivers generally have a very dynamic signature and are strongly dependent on time and space. The dynamic time course is due to the time- and space-variant input conditions resulting from fast overland (runoff and erosion, direct losses) and subsurface flow (artificial drainage), directly connecting surfaces and/or agricultural fields where pesticides are applied, to receiving rivers. In order to increase the effectiveness of mitigation measures a thorough understanding of pesticide behavior at the watershed scale and effective communication to farmers is needed. We developed a targeted approach where we first derived a map with priority zones for applying mitigation measures and used this information to communicate to local farmers with focus on those farmers with potentially a significant impact on the pesticide load to the river. A risk map representing the risk of pesticide runoff to the river was derived based on information about the topography, crop cover, the estimated pesticide use, the potential erosion risk, and the connectivity of the agricultural parcels to the river. Subsequently, the theoretical risk map was validated in the field using field observations of runoff during stormflow events, and using observations of roads short-circuiting the runoff to the river. From the validated risk map priority zones were defined for measures related to erosion control. The information was used to target farmers that may have a significant impact on the pesticide load to surface water. Those farmers were encouraged to participate in a voluntary erosion control program supported by the local government, starting from 2016 on. A strategy based on the installation of grassed zones located at the lower end of the cultivated fields (vegetated bufferstrips), in the major runoff pathways (i.e. grassed waterways) and the creation of (small) sediment depositions sites within the cultivated catchment is potentially very effective and efficient. Preliminary results show an additional 11 grass buffer strips have been installed in the catchment all in the priority zones. The effect of mitigation measures on water quality is further assessed in a monitoring campaign.

Nanomaterial fate and toxicity - Implications of the environment as a global reactor for nanomaterials along their life-cycle (II)

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Toxicity of multi-walled carbon nanotubes on the invasive clam Ruditapes philippinarum

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The production and use of carbon nanomaterials (CNMs), particularly Multi-walled carbon nanotubes (MWCNTs), is increasing rapidly and the need to assess their presence in the environment and associated risks has become increasingly important. Nowadays these new anthropogenic pollution does not occur isolated, acting in combination namely with climate change related factors. Although several studies demonstrated that ocean acidification may affect invertebrates, less is known on the influence of this environmental change on the bioavailability and toxicity of pollutants and on the sensitivity of invertebrates when exposed to pollutants, including CNMs. *Ruditapes philippinarum* has frequently been used to evaluate the impact of environmental disturbances in aquatic systems but, to our knowledge, no information is available on biochemical alterations induced in this species due to MWCNTs exposure under actual and predicted ocean acidification conditions. Thus, the present study aimed to assess the toxicity effects in *R. philippinarum* exposed to two pH levels (7.6 and 7.9-control) each one combined with different MWCNTs concentrations (0.10; 1.00 mg/L). Biochemical (oxidative stress related biomarkers, metabolic capacity and energy reserves) markers, after 28 days exposure period, were evaluated. The obtained results revealed that organisms under lower pH levels (7.6) showed similar response patterns to MWCNTs comparing to organisms under control pH (7.9). In fact clams exposed to MWCNTs increased their metabolic capacity (high electron transport system (ETS) activity) while using their energy reserves (decreased GLY, glycogen and PROT, protein concentrations), both under pH 7.9 and pH 7.6. Furthermore, under both pH scenarios clams exposed to MWCNTs increased the activity of superoxide dismutase (SOD), especially noticed at the highest exposure concentration (1.00 mg/L). However, although glutathione peroxidase (GPx) increased at the lowest exposure concentration (0.10 mg/L) the activity of this enzyme significantly decreased at the highest MWCNTs concentration, which was at least partially responsible for increased of lipid peroxidation (LPO). **Keywords**: Multi-walled carbon nanotubes (MWCNTs), pH variation, *Ruditapes philippinarum*, biochemical alterations

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Modelling emissions and fate of engineered cerium oxide nanoparticles at high spatial resolution in urban environments

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Urban environments require special attention when studying ENP transformation and transport as they include special reactors affecting contaminant speciation and fate compared to natural environments. Characteristics of urban environments, such as dominant impervious cover, decreased storm water infiltration and increased runoff among others and presence of technical reactors such as waste water treatment plants (WWTPs) will affect the overall fate of the emitted pollutants. However, the study of such emissions and the ENPs environmental behavior and fate mechanisms are particularly challenging due to limited data on ENP production and application patterns in cities and due to analytical limitations for their detection in environmental matrices. In this sense, models emerge as powerful tools to address these challenges by providing emission and concentration estimates, and by improving the understanding of behavior and fate processes through simulations of specific mechanisms in the different urban reactors. Here we present the results obtained from the application of a novel modelling approach that combines an emission and a fate model for ENPs with high spatial and temporal resolution for an urban environment (i.e. the city of York, UK). The model was applied to the study of cerium oxide engineered nanoparticle emissions when incorporated in fuel additives used by the bus fleet of the city of York and to their transport and fate in the rivers circulating along the city (the Ouse and the Foss). The model emission calculations where based on the local traffic patterns and sewage network connections obtained from different reliable and official sources such as Yorkshire Water and York City Council. Surface water characteristics acquired from an extensive and local monitoring campaign performed in the rivers Ouse and Foss helped to parametrize the river fate model. As a results, we are able to identify hot spots of cerium oxide nanoparticle emissions within the city and to study their transport and fate in the urban surface waters.

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Investigation on the mode of action of n-TiO2 with co-exposed Cd in the nematode test using Caenorhabditis elegans

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Even though, the use of synthetic nano-scale particles is rising steadily in consumer products and different industrial sectors, information on ecotoxicity of nanoparticles is still limited. This is especially true with regard to the interaction of nanoparticles with other contaminants that are potentially present in environmental matrices. This project focuses on the effect that co-occurrence of nano-scale TiO_2 (n-TiO_2) and cadmium have on nematodes, especially under simulated solar radiation (SSR). n-TiO_2 are probably the most environmentally relevant nanoparticles with accumulation rates in sediments in European rivers projected to be 1.4 mg*kg^{-1}*yr^{-1} (Gottschalk *et al*., 2007). Angelstorf *et al.* (2014) showed an increased toxicity of n-TiO_2 on the nematode *Caenorhabditis elegans* when irradiated by SSR, assuming interference with membrane integrity. As Cd is known to interfere with calcium channels, the impact of mixture toxicity on these channels was investigated, too. Single substance tests in the dark resulted in EC_{50} values (for reproduction) of 0.6 mg /L for Cd and > 200 mg /L for n-TiO_2. Supplementing n-TiO_2 suspension (40 mg/L) with 50 μg/L Cd inhibited reproduction twice more strongly compared to n-TiO_2 alone under SSR. Exposure to SSR led to a much more pronounced effect (80 % reproduction inhibition) in the mixture than would have been expected from single-exposures. This indicates an interactivity of modes of action of Cd and n-TiO_2 under SSR with consequences for the nematodes' life cycle. Studies are carried out to identify the underlying processes. [1] F. Gottschalk, T. Sonderer, R.W. Scholz, B. Nowack. 2009. Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions. Environ. Sci. Technol. 43:9216-9222. [2] J.S. Angelstorf, W. Ahlf, F. von der Kammer, S. Heise. 2014. Impact of particle size and light exposure on the effects of TiO_2 nanoparticles on *Caenorhabditis elegans*. Environ. Toxicol. Chem. 33:2288-2296.

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High-resolution mass spectrometry applied to the study of metabolome modifications in filter-feeding organisms after nanomaterials and microplastics administration through the diet

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During the last years, the occurrence of nanomaterials (NMs) in the environment and food has become an ecological and public health problem. Their occurrence has proved in different environmental compartments. However, little information has been related to marine environments while coastal areas and estuaries are suspected to be one of their major sinks. Despite of their low acute toxicity, there is a gap of information about their potential long-term effects at sub-acute concentrations although some recent studies revelead the ability to modulate the toxicity of other co-contaminants. Plastic debris pollution has been recognised as a significant problem in marine ecosystems. However, much of the recent concern has been focussed on microplastics (MPLs), but almost no data has reported the impact of nanoplastics (NPLs). Plastic particles are also known to adsorb persistent organic pollutants and a certain percentage of their weight may consist of additives, such as plasticisers. In addition, the presence and toxicological impact of MPLs and NPLs in estuaries and coastal areas needs to be addressed. In this study , the uptake of fullerene soot by primary producers and their potential scale-up by filter-feeders (*Mytilus galloprovincialis*) is studied at metabolomic scale by LC-HRMS; finally, the assessment of the the effects of the exposure of fullerene soot on *M. galloprovincialis* at sub-lethal concentrations and polyethylene have been evaluated. Primary results confirmed the uptake and scaling up of fullerenes through the aquatic trophic chain as well as significant metabolic response in target metabolites. The lowest concentrations of exposure (1.1 and 2.3 pg/l) mainly caused a slight increase in small non-polar amino acids such as branched-chain amino acids (leucine, isoleucine and valine) and the rise of glutamine and decrease of glutamic acid, which indicates a facultative anaerobiosis and starvation. Also, most of the concentrations of free amino acids were increased, which is in agreement with other studies that report the accumulation of high concentrations of some intercellular amino acids (e.g. proline) because they can be only degraded by enzymes under aerobic regime. Finally, levels of fatty acids were globally increased in a statistically significant manner. In the case of PE microplastics starvation effects produced by a decrease in filtration rates was detected, while oxidative stress or significant amino acids changes were not significant.

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Nonlinear learning systems for predicting nanoparticle toxicity
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Nanoparticle (NP) toxicity is determined by a vast number of topological, sterical, physico-chemical as well as biological properties, rendering *a priori* evaluation of the effect of NP on biological tissue as arduous as it is necessary and urgent. We aimed at mining the HORIZON 2020 MODENA COST NP cytotoxicity database through nonlinear predictive regressor learning systems in order to assess the power of available NP descriptors and assay characteristics in predicting NP toxicity. We analyzed multicentric data from 192 cytotoxicity experiments based on different assays (ATP, LDH and MTT+WST-1) in which several cell types were exposed to a number of nanomaterials for quantitative toxicity assessment. Selected prediction endpoints were EC25, EC50 and slope. In order to augment potential prediction capabilities, we tested and compared a variety of a kernel method known as support vector regressors (SVR), and in particular a e-SVR method, to a regression based on Radial Basis Function (RBF) networks. All experiments were performed within a nested-cross validation scheme for parameter optimization. In order to quantify the worth of an attribute in predicting the numerical (EC25, EC50) outcome, we both employed "best first" strategies as well as a greedy stepwise search method for feature selection. Most regressor learning systems achieved R2 values above 90% and the correlation between real and estimated toxicity endpoint values increased monotonically with the number of included features, reaching values above 0.90. Overall, toxicity endpoint EC25 was associated with the best prediction performance in both regressors. Searching the feature space according resulted in cumulative merit curves which also increased monotonically in most in silico experiments, and exhibited a near-plateau behaviour after inclusionof 4-6 features. Structural features as well as experimental conditions variables (e.g.time of exposure, primary size and aspect ratio) were consistently selected as the most relevant predictors. In this study, toxicity endpoints could be reliably predicted employing nonlinear regressor learning systems and only 5 to 6 simple structural and functional descriptors. This points towards the feasibility of a safe-by-design approach, in which specific attention is devoted to those features which are most predictive of toxicity.

Persistence & Biodegradation Assessment

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The impacts of light and season on isopyrazam degradation in river microcosms
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Industry uses tests designed by the Organisation of Economic Cooperation and Development (OECD) to assess transformation potential of a chemical in the environment before it can be approved for use. Various factors are important in determining chemical fate in the environment, but many are not considered in these tests. In particular, a range of environmental properties, such as light intensity, which can change seasonally, affects microbial abundance and diversity. OECD tests, however, are carried out in the dark, and there is no consideration of time of year that environmental samples are collected. We investigated the impact of light on the transformation of ^{14}C-isopyrazam, a fungicide, in river microcosms, using water and sediment collected at defined seasonal time points across a 2-year period. Materials were collected from the River Dene (Wellesbourne, UK), and both water and water-sediment microcosms were incubated under dark or light (16 hour light/8 hour dark cycle) conditions. Isopyrazam recovery was determined over a 36-day sampling period using HPLC. Additionally, microbial and water chemistry analysis was also carried out. There was little degradation of isopyrazam in dark microcosms regardless of season. In microcosms incubated under a light-dark cycle, however, degradation was faster in all seasons, although the rate of degradation varied depending on time of year sampling took place. Microcosms sampled in autumn or summer gave the fastest degradation rates, whereas microcosms containing samples collected in spring and winter gave slower degradation rates. Samples collected in winter showed variable degradation, likely due to variation in abiotic factors at the time of sampling. This trend was seen in both the water and water-sediment microcosms, although there was a greater effect in water-sediment systems. There were also differing degrees of mineralization across time, but the extent of mineralization was not correlated with the overall isopyrazam degradation rate. Profiling of microbial communities using 23S rRNA genes to amplify phototrophic taxa, and 16S rRNA genes to target heterotrophic taxa, demonstrated both treatment and seasonal shifts in community structure and diversity. This could account for differences in degradation rates across time points.

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Biodegradation testing of hydrophobic chemicals in mixtures at low concentrations - covering the chemical space of petroleum hydrocarbons
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Petroleum products are complex mixtures of varying composition containing thousands of hydrocarbons each with their own physicochemical properties and degradation kinetics. One approach for risk assessment of these products is therefore to group the hydrocarbons by carbon number and chemical class i.e. hydrocarbon blocks. However, the biodegradation kinetic data varies in quantity and quality for the different hydrocarbon blocks, hampering the characterization of their fate properties. In this study, biodegradation kinetics of a large number of hydrocarbons aiming to cover the chemical space of petroleum hydrocarbons, were therefore determined at ng/L to µg/L concentrations in surface water, seawater and activated sludge filtrate. Two hydrocarbon mixtures were prepared, comprising a total of 53 chemicals including paraffins, naphthenics and aromatic hydrocarbons from C8 to C20. Passive dosing from silicone rod loaded with the mixtures was used to prepare stock solutions. Test systems were then prepared using stock solution diluted with the surface water, seawater or activated sludge filtrate. Test systems were incubated at 20 °C on a roller for up to 98 days and analyzed using GC-MS and fully automated Solid Phase Micro Extraction. Results were normalized to parallel measurements of abiotic controls prior to evaluation of biodegradation kinetics. Degradation was generally faster in the activated sludge filtrate than in the seawater and lakewater. In the activated sludge filtrate lag phases were < 9 days for the 49 hydrocarbons that were degraded within test duration. Degradation rate constants and corresponding half-lives were determined for 44 of the hydrocabons. In lakewater and seawater, less test chemicals were degraded within the test duration compared to the activated sludge filtrate.

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Persistence of produced water compounds in seawater determined by two biodegradation methods
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The OSPAR Commission regulates operational discharges such as produced water (PW) from the North Sea offshore industry to the marine environment, classifying PBT substances according to the following criteria: Half-life > 50 days, BCF ≥ 500 or logPow ≥ 4, EC50/LC50 ≤ 1 mg/L or NOEC ≤ 0.1 mg/L. Among the discharged compounds from the water fraction of the reservoir, 2-6 ring PAHs and alkylated phenols (APs) cause environmental concern. Few studies have focused on the persistence of PW compounds after release to the marine environment. We developed two laboratory methods for determination of PW compound persistence

in seawater, using low concentrations of PW compounds. One method was based on immobilization of poorly water-soluble compounds to hydrophobic adsorbents (FluortexTM). This method enabled biodegradation studies both on the adsorbent surfaces and in the surrounding water. Three APs with suspected PBT properties were tested in the method (20°C; 64 days), and results compared to data from an OECD biodegradability method (OECD 306). While all three APs were classified as persistent (half-lives > 50 days) by the OECD method at high concentrations (2 mg/L), 2 of the 3 AP showed biotransformation half-lives < 50 days (determined by 1st-order rate kinetics) in the Fluortex method at low substance concentrations (79-116 μg/L). The second method consisted of a slowly rotating carousel system, in which PW effluents from a North Sea platform was mixed with seawater and incubated (13°C; 62 days). Compounds in the PW were separated between a particle-bound and a dissolved phase. Typical oil-mineral aggregation was observed in dispersions in natural seawater, but not sterilized controls. Analyses of PAHs and APs showed ≥ 90% biotransformation of naphthalenes and smaller PAHs (2- to 3-ring), as well as C0-C9 APs. Larger PAHs (4- to 5-ring) were degraded by 46%. Determination of single compound (30 PAH and 39 AP compounds) half-lives from first-order rate kinetics, showed half-lives < 50 days for 62 of the 69 PAH and AP substances. Studies have now been initiated to determine the acute toxicity to copepods of water-soluble fractions from hydrophic adsorbents (Fluortex) during biodegradation experiments, and to characterize metabolites in relation to toxicity changes during biodegradation.

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Degradation of synthetic polymeric flocculants in land spreading of MWWTP sludge
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The fate and effects of poly(acrylamide-co-choline acrylate) in soil was comprehensively investigated. In contrast to earlier studies the polymer was applied to soil as a component of dewatered MWWTP sludge in order to create a realistic exposure scenario. Degradability, leaching, uptake of degradation products and effects on soil microorganisms were studied in both standard laboratory experiments and outdoor simulations. Radiolabelled polymer was synthesized in the testing facility using ^{14}C-labelled acrylamide in a downscaled procedure with the labeling positioned along the carbon-carbon backbone of the amide units. The ^{14}C-labelled polymer met the product specifications of commercially available polymer used in sludge dewatering polymer. The ^{14}C-polymer was used to dewater digested sewage sludge from a local MWWTP in a standard way and the dewatered sludge containing the ^{14}C-polymer was used for the subsequent degradation tests. Degradation of the polymer in a real-life matrix was initially assessed using a modified OECD 307 test where the test duration was extended of one year and a second set of samples was exposed to simulated sunlight in a 12-hour day/night sequence. Subsequently, an outdoor simulation study was conducted in a Lysimeter device over three years. The fate of the polymer in agricultural soil was monitored along with frequent soil and eco-toxicological testing. This presentation focuses on the experimental methodology and in particular that of the analysis of a polymer in a complex environmental matrix. The project proved that even using a very conservative study design and evaluation method that the polymer complies with the requirements of German Fertilizer Ordinance (more than 20% degradation in 2 years) and has no adverse effect on the environment.

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Poster spotlight: MO142, MO143, MO144, MO145

Hazard and risk assessment of human pharmaceuticals in the environment

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Emerging micropollutants in Kenya: a case study on the occurrence patterns of pharmaceutical residues during wastewater treatment and in river water
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The technological revolution in the last decades has led to significant advancements in analytical sciences. As a result, it is now possible to detect trace organic compounds in the environment which were hitherto not considered as pollutants, such as pharmaceutically active compounds (PhACs). The frequent detection of PhACs in the environment coupled with the rising environmental consciousness globally has raised a lot of concern about their possible (ecological) effects. Despite a lot of environmental work being done on PhACs in the Western world, limited information is available from developing countries especially Africa. In this study, unique data on concentrations and loads of 25 PhACs (antibiotics, anti(retro)virals, analgesics, anti-inflammatory and psychiatric drugs) in two wastewater treatment plants and two rivers in Kenya are presented. Overall, drugs commonly used in combating common ailments in Kenya (e.g. HIV/AIDS and malaria) such as anti(retro)virals (e.g. nevirapine and zidovudine) and antibiotics (e.g. metronidazole, sulfamethoxazole and trimethoprim) were in all matrices highly prevalent (up to 167 μg L^{-1}). Both the overall removal (11–99%) in wastewater stabilization ponds (WSP, only scarcely studied regarding the removal of micropollutants) and the contribution of the different treatment steps showed large differences among the different PhACs. Some compounds were better removed in the WSP compared to conventional activated sludge systems. Compounds like paracetamol were removed up to below detection limit within the primary facultative ponds, while the maturation ponds showed to be more important for other PhACs. River samples collected in informal settlements and near waste dumpsites recorded similar or higher PhACs concentrations (up to 350 μg L^{-1}) than in untreated wastewater, an indication of their huge impacts on water quality in these areas. From this work, a distinct pharmaceutical contamination pattern is evident as compared to the Western world, a sign of a research gap which needs to be filled.

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Prediction of Drug Target Conservation in Wildlife Species
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There are approximately 1500 Active Pharmaceutical Ingredients (APIs) in use today and low level concentrations of hundreds of APIs are detected widely in the aquatic environment. Pharmaceuticals are designed to be biologically potent at low concentrations and therefore their presence in the environment, even at low levels, has raised some concerns. Moreover, most APIs in pharmaceutical products approved before 2006 lack chronic ecotoxicity data. Thus, methods are needed that can prioritise APIs and susceptible taxa for further testing. Species in which the drug targets and the pharmacological mode of action are conserved may be more susceptible to chronic pharmaceutical exposure. Conserved gene function across species can be predicted by inference of orthologous relationships. Various methods of ortholog prediction are available, each with different strengths and weaknesses. We have combined predictions from three well established databases Ensembl Compara, EggNOG and InParanoid to generate orthologous relationships between human drug targets and genes in wildlife species with higher accuracy and confidence than conducted previously. To facilitate the incorporation of drug target ortholog predictions in pharmaceutical ecotoxicity test design and interpretation, we have developed an online resource that provides an overview of available information in a user-friendly manner. We retrieved information on 1080 APIs targeting human proteins from DrugBank. These APIs targeted a total of 537 proteins, as defined by their UniProt ID. Orthologous relationships for the drug targets are presented in 644 species, 58 of which are shared across all three databases. Limiting our analysis to species present in all three databases and where at least two of the databases agreed on absence/presence of a drug target ortholog, 36 targets were found missing in all teleost fish and 5 APIs had all their targets in this group. For protostomes 354 APIs lack orthologous gene targets, meanwhile invertebrate deuterostomes may only lack targets for 167 APIs suggesting that *Daphnids* may not be the most appropriate invertebrate test species in some cases. Green algae, separated by a greater evolutionary distance, lack orthologs targeted by 74 % of the APIs. The resource presented will allow users to quickly gain an overview of the drug target conservation across a wide range of species, with significant value for hypothesis generation regarding susceptible species for API exposures.

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Unexpected Endpoints: Pharmaceutical 5α-reductase inhibitors, designed to treat prostate cancer in men, disrupt gastropod morphogenesis during embryo development.
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To protect the aquatic environment from pollution we need a sound understanding of how substances affect a range of aquatic organisms. Freshwater gastropod molluscs have recently been introduced into OECD chemical testing guidelines for reproductive toxicity. However, in comparison to vertebrates, we still have significant knowledge-gaps in fundamental developmental and reproductive mechanisms in these animals. For example, in mammals and other vertebrates, 5α-reductase (5αR) converts the steroid androgen, testosterone (T) to the more potent dihydrotestosterone (DHT). In vertebrates', such as frogs and fish, disruption to 5αR during development is known to compromise male reproductive

function later in life, without overt toxicity or morphological changes. 5αR homologs have also been identified in molluscs, however recent findings suggest that the freshwater gastropod *B. glabrata* does not use vertebrate steroid androgens in their reproductive development, and genomic searches have shown they do not possess an androgen receptor. This suggests an, as yet, unknown alternative function of 5αR in gastropods. The aim of this study was to investigate if 5αR enzymes have a physiological role in gastropods. Here we use selective pharmaceutical inhibitors of 5αR: dutasteride (DUT) and finasteride (FIN), as tools to explore 5αR's function during *B. glabrata* embryo development. These compounds elicited a strong, highly reproducible phenotypic response inducing elongated 'banana' shaped shell morphology, suggesting 5αR enzymes do have a physiological role in gastropod development. Our results show that the morphological developmental effects induced by dutasteride and finasteride in *B.glabrata* occur in a dose dependant manner, and at similar concentrations (μg/L) to those reported to impact aquatic vertebrate reproductive development. We also demonstrate that, similar to vertebrates', in *B. glabrata*, dutasteride is a more potent 5αR inhibitor than finasteride. As yet we do not fully understand the role of 5αR enzymes in molluscs, or the molecules and pathways involved in producing this elongated phenotype. However, it highlights that the physiological endpoint of chemical disruption can be significantly different between model test species, i.e. fish and gastropods. More research is required to better understand fundamental biology of molluscs if we are to protect them from pollution in the environment.

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How biotransformation can make the heart beat - mixture effects of two pharmaceuticals (metoprolol and paroxetine) in the zebrafish embryo

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The zebrafish embryo (ZFE) is increasingly employed as a test organism in ecotoxicology and toxicology. As shown recently biotransformation in ZFE can occur already in its early stages of development and transformation rates can be high enough to affect the internal concentration of the test compound [1]. In this study it was tested (a) whether biotransformation can also modulate biological effects of test compounds in ZFE and (b) whether this is affected if a second test compound interferes with biotransformation. The β_1-blocker metoprolol and the selective serotonin reuptake inhibitor paroxetine were used as test compounds. ZFE were exposed to metoprolol alone or in combination with paroxetine at different concentrations. Paroxetine is known to inhibit the enzyme Cyp2D6 in mammals [2]. Transformation products (TPs) were detected and identified by LC-HRMS, whereas internal concentrations of metoprolol and its three major phase- I TPs were determined by LC-MS/MS. Heartbeat was determined visually using a microscope. The internal concentration of metoprolol reaches its maximum around 72 h of exposure. With the help of liquid chromatography-high resolution mass spectrometry we detected and tentatively identified 22 TPs of metoprolol, belonging to phase-I and, less so, to phase-II metabolism. When ZFE were exposed to metoprolol and paroxetine the internal concentration of metoprolol was significant higher and the concentration of TPs was significantly lower. This suggests that the ZFE uses an enzyme system for metoprolol degradation that can be inhibited by paroxetine and which might be seen as an ortholog to the mammal Cyp2D6. However, the co-exposure to paroxetine did not only increase the internal concentration of metoprolol, but it also decreased the heartbeat of the exposed ZFE. This is a very illustrative example of a mixture effect, where one chemical affects the biotransformation activity of the organism and, thereby, increases the biological effect of a second chemical. [1] Brox S, Seiwert B, Kuester E, Reemtsma T. (2016) Toxicokinetics of polar chemicals in zebrafish embryo (*Danio rerio*): Influence of physicochemical properties and of biological processes. Environ Sci Technol 50, 10264 – 10272. [2] Hemeryck A. and Belpaire F.M. (2002) Selective serotonin reuptake inhibitors and cytochrome P-450 mediated drug-drug interactions: an update. Curr Drug Metab3, 13-37.

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Review of the EU guideline on ERA of HMPs

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The 'Guideline on the environmental risk assessment of medicinal products for human use' (EMEA/CHMP/SWP/4447/00, rev. 2) was adopted in 2006 and supplemented with a 'Questions and Answers' document in 2011 (EMA/CHMP/SWP/44609/2010, rev. 1). In 2016 the European Medicines Agency has initiated a revision of the guideline. The revision is based on a concept paper (EMA/CHMP/SWP/65429/2016) which was also subject for public consultation. Eight topis are outlined in the concept paper which will be addressed in the revision of the guideline. The presentation will present the view of the German Environment Agency (UBA) on issues of the ERA of human medicinal products which are often controversially discussed. One aspect adressed in the concept paper is a review of the tiered approach strategy and triggers for further assessments and additional studies: The key element of the current tiered ERA approach is the action limit of 0.01 μg/l triggering an in depth ERA based on experimental data on environmental fate and effects. This PEC action limit is controversially discussed. There is a demand to substanciate the scientific base of this value in the light of all available data. However, there are some substances as e.g. endocrine active substances, for which the action limit may not be applicable. UBAs proposal to deal with this issue in future is to skip the PEC action limit totally and to replace the current Phase I assessment by a set of yes/no-questions to filter active substances for which an in depth ERA is required. There are also other values given in the guideline triggering further assessment. Most of them are well established and also used in other regulatory frameworks. However, there might be a need to reevaluate some of these values and their applicability for pharmaceutical substances. An other aspect, which is discussed for years is how to use the known pharmacodynamic and pharmacokinetic properties of pharmaceuticals in the ERA. The current guideline resp. the questions and answers document already provides some guidance on how to tailor an ERA for endocrine active substances. However, some other examples of pharmaceutical substances exist for which a more tailored risk assessment could be useful. One of the main challenges of the guideline review will be to find a balance between a clear guidance without misleading options for interpretations and providing options to tailor the ERA according to the specific properties of the pharmaceutical substance.

LCA of territorial contexts: upscaling the Life Cycle Thinking to business clusters, neighborhoods, urban agglomerations and territorial entities

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Life Cycle Assessments applied to the urban scale: a review of selected literature and critical analysis of current trends and limitations

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Cities are the major poles of aggregation of human beings and, for the first time in human history, more than 1 person out of 2 is living in urban area. As cities concentrate also most of the world's economic activities, this convergency of human and economic capital could lead to higher environmental pressures on planet Earth, if not properly addressed. This means that holistic methodologies are necessary to understand, manage and tackle this challenge for the urban contexts, such as Life Cycle Thinking (LCT) and, specifically, Life Cycle Assessment (LCA). Neverthless, the application of LCA to the whole urban scale is not a reality yet. The aim of this contribution is to provide a comprehensive review of the existing literature in the field of LCA applied to the whole urban context, published from 2010 to the present, analyzing: i) the application of LCA for the different urban sub-sectors; ii) furher combination of LCA with other top-down methodologies and technological tools; iii) upscaling approaches. The output of this review shows that few applications of LCA at the whole urban scale exist to date. Waste and water sub-sectors look to be the most mature in performing the transition from the product/process level to higher scale systems. Tranportation sectors and energy systems follow, mainly to assess and compare emissions and energy demands or technology systems, respectively. For household consumption and the built environment no pure LCA analysis is available now. A unique attempt to fuse LCA with UM at the whole urban scale exists, while the integration with other methodologies is reported to be still limited in scope when applied to the urban context. A claim for integration of LCA with spatial planning and ICT tools is worth to be mentioned. Finally, first attempts of upscaling approaches are available in literature. The most comprehensive is the Territorial LCA, while others focus on a specific urban issue and/or they are applied to a limited spatial extent, e.g. neighbourhood scale. The lack of application of LCA at the full urban scale shows that further adjustments and improvements to the methodology are needed. Most of the applications to the different urban sectors are still immature for a transfer from product/process level to system levels. Several issues are still open: i) definition of system boundaries, ii) collection and management of the life cycle inventory; iii) more specificity in the impact assessment.

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Upscaling building LCA to neighbourhoods

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In order to move towards a sustainable built environment, modern cities need to be planned and organized differently, focussing not only on the characteristics of buildings but also on the relation between urban morphology, built density and the required infrastructure. However current Life Cycle Assessment (LCA) tools in the building sector are mostly limited to the assesment of individual buildings. This paper proposes an approach to upscale building LCA to the neighbourhood level by including the impact of infrastructure works such as transport networks, utilities and open spaces. To deal with the complexity a hierarchic assessment structure, using the principles of the "element method for cost control" and a subivision in different scale levels is implemented. To illustrate the methodology two schematic neighbourhood models with a diverse built density are analysed, consisting respectively of detached and terraced houses. Next to the buildings, the environmental impact of the roads, footpaths, parking facilities, square, piped and electrical services is assessed. The results reveal that the life cycle environmental impact of the model with terraced houses is about 15% lower than of the model with detached houses. The contribution of infrastructure works to the total neighbourhood impact, ranging from about 10% to 20%, is lower than the contribution of buildings but not negligible especially in low built density neighbourhoods. The comparison highlights the importance of optimizing the neighbourhood layout and built density to reduce the environmental impact of the built environment.

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Using and adapting the concept of eco-efficiency to assess land use planning scenarios in territorial LCA
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Although Life Cycle Assessment (LCA) was initially designed to assess the environmental impacts of a product or a service, recent proposals have been made to broaden the object of analysis in LCA towards meso-level systems such as cities or territories. The LCA framework has been adapted to assess territorial systems both for baseline analysis and for comparison of the environmental performances of different land use planning scenarios. One of the main adaptations of the resulting approach, named territorial LCA, relies on the definition and quantification of a set of services or land use functions provided by a given land planning scenario implemented on a territory. At the end, territorial LCA allows to compute the eco-efficiency of the studied scenario defined by the ratio of the economic (or social) value of the services provided by the land use planning scenario to its environmental impacts. The aim of this work is to assess the eco-efficiency of different land use planning scenarios elaborated on a French mediterranean case study, and to discuss the interest and limitations of this indicator according to its links with relative and absolute environmental sustainability. Three contrasting land use planning scenarios are defined. The first one is businal as usual. The second one supports agricultural and industrial developments while the third one is based on residential economy. By implementing the territorial LCA framework, different eco-efficiency ratios can be computed for the three scenarios. For instance, the comparison of the eco-efficiencies defined by the ratios of the job creation value to the impacts of all production activities shows that the third scenario is the most performant. However, this result is not sufficient to assess absolute environmental sustainability. The use of carrying capacity based normalisation references (CCNR) show that for certain environmental issues such as climate change, none of the scenarios is below environmental thresholds, no matter the valuation principle chosen to compute this reference. This means that eco-efficiency analysis should integrate information on carrying capacity. For instance, CCNR values could be used as fixed values in the denominator of the eco-efficiency ratios when defining scenarios. However, work is still needed to compute CCNR adapted to territorial systems by considering their spatial variability and their levels of self-sufficiency for the provision of services.

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LCA in support to urban planning policies
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It is now recognized that urban scale actions in the transport, building and urbanism sectors are essantial to achieve climate change mitigation. Initially dedicated to the evaluation of products and services, life cycle assessment (LCA) is more and more used to evaluate environmental impacts of complex systems like neighborhoods, building stocks or territory. Yet, prevalent challenges remain for the application of LCA at city scale. First is the definition of city's life cycle itself. Cities are dynamic and in constant evolution systems, the concept of life cycle cannot be applied to them. Then, on the operationnal perspective, LCA requires the system to be modelised in details. At urban scale, this means a large amount of data is necessary. To overcome the first issue, this study develops the coupling of material and energy flow analysis (MEFA) with LCA. Regarding the operational challenge of data collection, it is recommended the use of (GIS) tools to simplify flows accounting. The urban area of Toulouse (France) is used as a case study to test the methodology. Two alternatives for the city evolution for the period 2010-2100 are assessed. Prospective scenarios and related GIS prospective maps established in previous research are used to run automated simulations. First, material and energy flows are estimated for each urban prospective scenario taking into account input and output flows of materials and energy for each building construction, use phase, renovation and demolition. Then, environemental impacts of material and energy flows are calculated using LCA. This study aims at developing operational solutions to support local authorities in urban planning decisions.

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An application of Spatial Territorial LCA to agriculture in Luxembourg: use the TMML R package
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Nitschelm et al. (2016) recently developed a method termed spatialized territorial LCA (STLCA) that combines spatialized LCA and territorial LCA to study land-use planning in an agricultural territory, focussing on the trade-off between agricultural production and the environment. Although this method would allow the integration of environmental impacts of human activities within the concept of environmental health inequalities, Nitschelm et al. (2016) remark that "the method needs to be tested using real case studies to determine its applicability". We present here the package called "Tailor-Made Machine Learning" (TMML) that we have developed in the software R to facilitate the application of the STLCA methodology. Currently the package comprises a SpatialPolygonsDataFrame describing fertilizers emissions of seven substances into different environmental media in the territory of the 116 administrative communes of Luxembourg and the basic functions for statistical cartography and spatial clustering allowing the representation of environmental health inequalities. The presentation will illustrate the application of the main functions of the package and show more extensive results for the case of Luxembourg agricultural surface. The release of the package will certainly facilitate the implementation of the methodology, thus helping LCA experts with the generation and of meaningful spatially differentiated result to help territorial governance. **Keywords:** spatialized territorial LCA; tailor made machine learning, agricultural emissions; environmental health inequalities

The role of ecotoxicology in fisheries science and aquaculture (II)

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Do fisheries practices increase contaminant mobilisation from sediments?
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Sediments often act as sinks for hydrophobic organic contaminants in the world's oceans. While commercial benthic trawling is estimated to affect around 15 million km^2 of the world's seafloor every year, only few studies have investigated whether this disturbance remobilises sediment-associated contaminants and, if so, whether these are bioavailable to aquatic organisms. Such regular practice in the Grenland fjord system in the south of Norway has the potential to affect the fate, movement, and bioavailability of sediment-associated polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs). This field study undertaken in this trawled contaminated Norwegian fjord showed that a single 1.8 km long trawl pass created a 3–5 million m^3 sediment plume containing around 9 t contaminated sediment; ie. 200 g dw m^{-2} trawled, equivalent to c. 10% of the annual gross sedimentation rate. Passive sampling devices consisting of towed semipermeable membrane devices attached to the trawl net were able to detect significant increases in the freely dissolved concentration of PCDD/F in the resuspended plume of sediment. PCDD/Fs from the sediments were also taken up by transplanted mussels which, during one month, accumulated them to levels above the EU maximum advised concentration for human consumption. We expect regular trawling in this fjord will likely cause a semi-permanent contaminated sediment suspension to be present in bottom waters.

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Environmental contaminants in wild fish from the North East Atlantic
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The North East Atlantic Ocean is a highly productive Oceanic area, with high biological productivity fuelled by the warm Gulf Stream. The ocean sustains major fisheries and thereby important food resources for a growing human population. Norway alone harvest around 2.5 million tons of wild seafood species (including molluscs) from these areas in addition to 1.3 million tons of farmed fish, mainly salmon, farmed in the same ocean. Several toxic substances do however also end up in the same ocean areas, both from local sources and to a large extent through long-range transport through air and by water currents. Even though diluted through travel, they might pose a potential harm to both humans and the marine life due to bioaccumulation. Our research has mainly been focused on large volume species such as herring (*Clupea harengius*), mackerel (*Scomber scombrus*), cod (*Gadus morhua*), saithe (*Pollachius virens*) and haddock (*Melanogrammus aeglefinus*). However, our research priorities have recently also been influenced by risk assessments regarding which species are more prone to accumulate toxic substances on which deep-water species, especially Greenland halibut (*Reinhardtius hippoglossoides*) and tusk (*Brosme brosme*), have been species of high attention. The main emphasis has been on substances with a risk of being close to tolerable weekly intake limits (TWI or PTWI) developed by EFSA or JECFA or maximum levels established by the EU. The substances with the highest potential in

this matter is heavy metals such as mercury and cadmium as well as POPs such as dioxins and PCBs. During the last 11 years, we have sampled and analyzed a large number of fish from the North Sea, the Norwegian Sea and the Barents Sea. This includes large baseline studies as well annual monitoring of some indicator species. Our analytical data on seafood species are actively used in the Norwegian Ocean management plans, and data on toxic substances in seafood data is a descriptor in the EU Marine Strategy Framework Directive's evaluation of environmental status. Major research areas are to explain levels of toxic substances in terms of species differences, spatial variations, size variation and temporal changes. There is a strong geographical variation, generally with increasing levels from North to South and from open sea to coast and fjord areas. The North Sea cod shows three times the mercury level of the Barents Sea cod and similar patterns are found in saithe and tusk. For the pelagic species, such patterns are not that clear while seasonal variations based on maturation and spawning cycles are important. We find that liver of lean fish in coastal areas often exceed the maximum level for dioxins and dioxin-like PCBs in a range of species. However, the general patterns are not without interesting exceptions. The edible crab (*Cancer pagurus*) is an appreciated food item in several countries, and its fishery is of significant economic value in many European countries. The brown meat of the crab is possibly the marine food, which can accumulate the highest levels of cadmium. The highest levels were, however, observed in Northern Norway, with little data to facilitate an understanding of the higher levels. Based on the generally accepted TWI/PTWI values, mercury will be the limiting factor for high seafood intake for fillet of most fish species. A large halibut or a tusk from a heavy metal contaminated fjord can easily reach 1.5 mg Hg/kg w.w., and in those cases, only one meal per month may fill up the TWI for methyl mercury. While for other species such as cod from the Barents Sea, it would take 16 meals per week to reach TWI. We have built an extensive monitoring program of wild seafood to obtain the necessary data which may keep seafood with illegal levels of contaminants away from the consumers, and which should make our food safety authorities more able to prevent elevated intake of toxic substances. However, which new substances and species to include in the monitoring, is a matter for continuous discussion.

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Parameters influencing the levels of emerging contaminants in seafood from estuaries and open seas
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In this presentation results of a large monitoring programme, as part of a European Research project (ECsafeSEAFOOD) as well as of yearly monitoring programmes of Wageningen Marine Research will be shown. Factors that determine the levels in different species were investigated. The focus was on non-regulated emerging contaminants, many of those have an origin in human activities. Therefore, fish (flounder, mullet) and shellfish (mussel, clam) from potential hotspots like the Western Scheldt and Ebro were fist monitored for a wide variety of organic contaminants, including pharmaceuticals, UV filters, musks, PFAS, brominated flame retardants as well as arsenic and mercuric species. Only contaminants detected above LOQ were later monitored in many, commercially important, seafood species. These species were sampled at two sizes when appropriate, at several places and in two rounds, from European seas, but also from the Atlantic and Pacific Ocean as well as from aquaculture. Commercially important seaweeds were sampled for metals also. From this large amount of samples factors that drive concentrations of contaminants in seafood were determined. Not surprisingly, location was the main parameter. Moving from a hotspot to open seas concentrations of contaminants dropped quickly. Similarly, commercial species obtained from coastal areas tend to be more polluted than species from open seas. Yet, sometimes human-made contaminants were observed in open seas at specific locations only, with no known history of human activities. The sampling itself, as shown by the two sampling rounds, also contributed to the observed levels; very large differences in levels of some contaminants were observed between consecutive samplings (autumn and spring). The type of species, as well as the size, also influenced the levels of some contaminants. For mercury, size does matter considerably, as demonstrated before. However, species specific ecology could result in higher levels of mercury in smaller individuals. For other, less bioaccumulating contaminants the effect of size and species was less pronounced. As the intake of bioaccumulating compounds in farmed fish is dominated by the feed given, the difference in levels of contaminants clearly showed that fish feeds used were not of the same quality. **Acknowledgments** The research leading to these results has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 311820 (ECsafeSEAFOOD).

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Has tributyltin contamination reduced catches of the brown shrimp Crangon crangon?
K. Cooreman, ILVO

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Discussion

Toxicology and ecotoxicology: bridging the gaps (Part II: models and frameworks)

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From Adverse Outcome Pathways to chemical risk assessment
A. Boobis, Imperial College London / Centre for Pharmacology & Therapeutics
Studies on adverse outcome pathways (AOPs) (aka modes of action) have played a key role in determining the relevance of effects observed in experimental animals to humans, in interpreting dose-response relationships and interindividual variability and in biomarker development. However, this is largely reactive – explaining the biological significance of adverse effects observed in experimental animals, i.e. a 'top-down' approach. In addition, throughput is low. As a new generation of *in silico* and *in vitro* methods is developed, where perturbations of biological pathways leading to adverse responses are identified and effects *in vivo* are predicted using physiologically-based models, AOPs have a key role to play in the design of such methods. The nature and magnitude of the effects studied should be relevant to potential adverse outcomes, based on prior knowledge on the toxicological significance of the AOP, and thus it should be possible to postulate a plausible adverse outcome pathway, i.e. a 'bottom-up' approach. However, for these approaches to fulfil their full potential, there is need for an agreed strategy for establishing their fitness-for-purpose: what is their qualitative and quantitative reliability, relevance to adversity, adequacy in assessing all potential toxicity, how much human variability is captured. Progression to their use in quantitative risk assessment is likely to require a more systems-based approach, whereby quantitative information on biological processes, PK and metabolism is integrated to provide a more accurate prediction of the adverse effects of a compound. The key events in an AOP can provide the 'building blocks' in such models, to enable quantitative dose-response characterization and population variability assessment in adverse outcomes.

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Application of Adverse Outcome Pathways (AOPs) in Human Health and Ecotoxicology – Capturing Divergent Consequences of Conserved Molecular Initiating Events via AOP Networks
D.L. Villeneuve, U.S. EPA / National Health and Environmental Effects Research Laboratory
The adverse outcome pathway (AOP) framework was developed to help organize and disseminate existing knowledge concerning the means through which specific perturbations of biological pathways can lead to adverse outcomes considered relevant to risk-based regulatory decision-making. Because many fundamental molecular and cellular pathways are conserved across taxa, data from assays that screen chemicals for their ability to interact with specific biomolecular targets can often be credibly applied to a broad range of species, even if the apical outcomes of those perturbations may differ. Information concerning the different trajectories of adversity that molecular initiating events may take in different taxa, life stages, and sexes of organisms can be captured in the form of an AOP network. As an example, AOPs documenting divergent consequences of thyroid peroxidase (TPO) and deiodinase (DIO) inhibition in mammals, amphibians, and fish have been developed. These AOPs provide the foundation for using data from common in vitro assays for TPO or DIO activity to inform both human health and ecological risk assessments. They also provide the foundation for an integrated approach to testing and assessment, where available information and biological understanding can be integrated in order to formulate plausible and testable hypotheses which can be used to target in vivo testing on the endpoints of greatest concern. Application of this AOP knowledge in several different risk contexts will be illustrated. Furthermore, the example will be used to highlight both the utility and limitations of the AOP framework and suggest additional 21st century tools that must be developed to facilitate the vision of a more predictive approach to ecological and human health risk assessment. *The contents of this presentation neither constitute, nor necessarily reflect, US EPA policy.*

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The zebrafish embryo model for human risk assessment: a SWOT analysis
S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology, Dept Veterinary Sciences
During the last 2 decades zebrafish (*Danio rerio*) embryos gained a lot of interest as a replacement of animal studies for toxicity testing in the EU since they are non-protected organisms until the free-feeding stage (i.e. 120 hours post fertilization (hpf)). So far, the fish embryo acute toxicity test (FET) is the only validated alternative, i.e. for environmental risk assessment of chemicals. However, as zebrafish organogenesis is very similar to organogenesis in higher vertebrates,

including humans, this species is also used for teratogenicity screening of pharmaceuticals. This may be as a stand-alone assay or part of a testing battery depending on the policy of the pharmaceutical company. Compared to other alternatives for teratogenicity testing, such as the mouse embryonic stem cell test (mEST) and the rat whole embryo culture (rWEC), the major advantage of the zebrafish embryo model is that it covers the whole period of organogenesis "*in vivo*". Furthermore, zebrafish embryos are transparent and develop externally, which facilitates chemical exposure, manipulation and evaluation of several morphological and behavioral endpoints in the intact living embryo. In general, several studies show a high concordance (80-85%) between the findings in the zebrafish embryo assay compared to the *in vivo* developmental toxicity studies in mammals, in which pregnant dams are exposed during organogenesis and their fetuses evaluated after C-section. Therefore, the zebrafish embryo assay is currentlyfurther explored as a potential replacement for one of the *in vivo* regulatory developmental toxicity studies in mammals. However, the false positive and false negative results reported in literature should first be further investigated as several factors, such as the study design (strain, start of exposure, morphological parameters etc.) and bioavailability of the compound (uptake, physicochemical properties), but also the biotransformation and bioactivation potential of the zebrafish embryo may confound the results. Indeed, although the duration of exposure (96 h) is very similar to the FET, the protocol for developmental toxicity assessment is much less standardized, which complicates the interpretation and extrapolation of the results. From the above, it is clear that the model requires further characterization and identification of its potential limitations prior to starting rigorous validation efforts for human risk assessment.

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The fish embryo model for the environmental assessment of chemicals
M. Leonard, lOREAL SA
There is a worldwide trend to promote the use of alternative to animal testing for the human and environmental safety assessment of chemicals. It's even required by certain authorities for the assessment of cosmetic products and ingredients. Fish are key aquatic models in environmental risk assessment but fall into the scope of several international regulations for the protection of animals used for scientific purposes. As a consequence, the fish embryo model (mostly zebrafish and medaka) provide an ethically acceptable alternative In compliance with international animal welfare regulations. So far, only the (zebra)fish embryo test [(Z)FET], which assess acute toxicity of chemicals, has been adopted as an international guideline validated under the umbrella of OECD (OECD TG n°236). In this assay, newly fertilized zebrafish embryos are exposed to the test chemical for a total of 96 h and the evaluation is limited to four endpoints related to lethality, i.e. coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail bud from the yolk sac and lack of heartbeat. The regulatory acceptance of the OECD TG n°236 is still debated at the European level, but countries such as Germany have already implemented their regulation to replace the fish toxicity assay for the assessment of effluents, by a simplified version of the fish Embryo test. In addition, when sub-lethal endpoints such as developmental malformations, malfunctions or behavior abnormalities are added to the fish embryo test, its prediction of longer term adverse effects is significantly improved. Integrating markers of specific AOPs may optimize the Fish embryo test and make it promising to anticipate the outcome of standard fish chronic toxicity test such as the OECD TG 210.

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Panel discussion

Alternative approaches to animal testing for (eco)toxicity, and the regulatory application of the 3Rs in chemical risk assessments (I)

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Development of an AOP describing effects of narcotics on membrane-bound mitochondrial processes in fish
L. Vergauwen, E.D. Michiels, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; E. Stinckens, University of Antwerp; S.N. Schmidt, P. Mayer, Technical University of Denmark / Department of Environmental Engineering; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Center; G.T. Ankley, U.S. EPA / National Health and Environmental Effects Research Laboratory; R. Blust, University of Antwerp; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences
Around 70% of industrial chemicals are hydrophobic compounds which are assumed to cause toxicity through narcosis by disrupting membrane integrity and function. The mechanistic details of how these chemicals cause toxic effects at the molecular and cellular level are largely unknown. Alternative tests including *in vitro* and zebrafish embryo tests could aid in classifying chemicals as narcotics and predicting toxicity complementary to quantitative structure activity relationships. In order to select the correct assays, we need more information on the mechanism of narcosis. We built an AOP network based on our hypothesis that the cell membrane is the first target of lipophilic compounds, which may then further partition into different organelle membranes where they can disrupt essential membrane-bound processes, such as the mitochondrial electron transport chain (ETC). We exposed zebrafish embryos to three narcotic compounds with increasing lipophilicities: 1,3,5-trichlorobenzene, phenanthrene, and pentachlorobenzene using a passive dosing method. To assess events along the hypothesized mitochondrially-related AOP, we measured electron transport chain activity, heart rate, swimming performance, growth and survival. The combined effects on ETC activity and other endpoints suggest that the initial increase of ETC activity at low exposure concentrations was due to a compensatory response. With increasing exposure concentration, ETC activity decreased coinciding with decreasing heart rate, increasing occurrence of malformations and severely decreased swimming activity indicative of a breakdown phase. Eventually ETC activity decreased below control levels at high exposure concentrations where we observed failure indicated by mortality and a 20% decreased heart rate. Additionally, we found that the maximum observed ETC compensation was log K_{ow} dependent. This relationship suggests that ETC compensation is an important characteristic of narcosis. Our data support the hypothesis that the electron transport chain is affected by narcotics. We also observed most of the adverse outcomes stipulated in the hypothesized AOP network, namely reduced survival, growth, heart rate and swimming activity. Currently, we are investigating whether narcotics directly affect electron transport chain complexes in *in vitro* tests, and whether the observed changes in cellular respiration result in changes in respiration at the organismal level.

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An improved spg1-gfp medaka assay for the detection of (anti-)androgens - the RADAR assay
A. Tindall, G. Christien, Watchfrog S.A.; I. Katsiadaki, Cefas / Environment and Animal Health; B. Demeneix, MNHN; G. Lemkine, Watchfrog S.A.
Over the last decade or so the prevalence of androgen axis disruptors and in particular anti-androgens has become apparent, in particular among pesticides, with *in vitro* studies identifying 66/200 and 37/134 pesticides tested as anti-androgenic [1,2]. However, the effects of many of these pesticides on the androgen axis have yet to be confirmed *in vivo*. In addition to ethical concerns regarding large scale *in vivo* screening campaigns, a major hurdle has been the lack of a medium throughput *in vivo* assay for androgen axis disruption. We developed a transgenic medaka line harbouring a portion of the *spiggin1* gene promoter driving expression of GFP. We previously demonstrated that this line is capable of correctly identifying androgens and anti-androgens, including pesticides, with a similar sensitivity to the androgenised female stickleback screen (AFSS) but in a greatly reduced time frame. The Rapid Androgen Disruption Animal Replacement (RADAR) assay provides a reliable medium throughput tool for screening potential EDCs acting on the androgen axis. In addition, this model, based on the use of early life stages, not considered as laboratory animals, also complies with the three R's principle of animal replacement. Finally, this improved protocol greatly reduces the number of fry required per test, offering additional ethical advantages. Here we describe recent improvements in the test protocol itself in addition to improvements related to the use of homozygous fry harbouring a transgene coding for a red eye marker cassette on the same chromosome as the *spiggin1-gfp* transgene. These improvements have led to an increased sensitivity, an increase in robustness and a decrease in the number of fry required per test. Finally, we show that this line is compatible with effect directed analysis and discuss recent progress towards OECD validation.

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In vitro function of the aryl hydrocarbon receptor predicts in vivo sensitivity to dioxins among all oviparous vertebrates
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More than 200-fold differences in concentrations of dioxin-like compounds (DLCs) that cause adverse effects on embryos have been observed among species of vertebrates. DLCs, which include polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs), share similarities in structure and bind with great affinity to the aryl hydrocarbon receptor (AHR). Activation of the AHR by DLCs regulates adverse effects associated with exposure to DLCs in vertebrates. Birds and fishes express two AHRs, an AHR1 and an AHR2. Prior investigations demonstrated that sensitivity to activation of the AHR1 (EC_{50}), but not AHR2, in an *in vitro* luciferase reporter gene (LRG) assay using transfected COS-7 cells was predictive of the sensitivity of embryos (LD_{50}) across all species of birds for PCDDs, PCDFs, and planar PCBs. However, nothing was known regarding whether sensitivity to activation of either AHR1 or AHR2 by DLCs is predictive of sensitivity to DLCs of embryos of the largest group of vertebrates, the fishes. Therefore, the aim of this study was to elucidate the role of the AHR1 and AHR2 in the observed differences in sensitivity among phylogenetically diverse species of fish. Specifically, this study investigated *in vitro* sensitivity to activation (EC_{50}) of the model DLC, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) of AHR1s and AHR2s among eight species of fish of known sensitivity of embryos to TCDD. There was no statsitically

significant linear relationship between *in vitro* sensitivity of AHR1 and *in vivo* sensitivity among the investigated species (R^2=0.33, p=0.23). However, there was a highly significant linear relationship between *in vitro* sensitivity of AHR2 and *in vivo* sensitivity among the investigated species (R^2=0.96, p=< 0.0001). The slope and *y*-intercept for the linear relationship for AHR2 of fishes is not statistically different from the slope and *y*-intercept for the linear relationship for AHR1 of birds.The relationship between *in vitro* sensitivity of AHR1 and *in vivo* sensitivity among birds is well established across PCDDs, PCDFs, and planar PCBs. Therefore, all *in vitro* and *in vivo* data for fishes and birds across all DLCs was combined into a single highly significant linear relationship (R^2=0.87; p=< 0.0001). It is anticipated that this linea relationship can be used to predict the *in vivo* sensitivity of any species of oviparous vertebrate to any DLC for application to ecological risk assessment.

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Development of high-throughput in vitro bioassays for determination of the CYP450 -inducing potential using chemically-defined media and suspension cell culture

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The application of *in vitro* test systems to reduce or replace animal experiments is an excellent example of the 3Rs principle. With the application of *in vitro* technology, various *in vivo* test systems for activity (drug candidates) and toxicity could be replaced with cell-based *in vitro* test systems. However, these *in vitro* techniques can be further improved by means of efficiency, reproducibility and sustainability. One aspect that leaves room for optimization is the culture medium, which is essentially supplemented with complex animal-sourced components. Especially, the fetal calf serum is a cost and quality determining factor with massive ethical concerns. The application of *in vitro* bioassays with permanent cell lines adapted to animal component-free, chemically-defined media in suspensions is a way to overcome this problem. In this proof-of-concept study, we present the three analytic liver cell lines ewoCL^H, ewoCL^R, and ewoCL^Z adapted to a single chemically-defined, serum-free medium as well as suspension culturing conditions. These cell lines were derived from liver cells lines originating from human (HepG2), rat (H4IIE) and fish (ZFL), respectively. With these cell lines bioassays for CYP450 activity and induction were conducted. The assays included enzymes like 7-ethoxyresorufin-*O*-deethylation (EROD), 7-pentoxyresorufin-*O*-dealkylation (PROD) and 7-benzyloxyresorufin-*O*-dealkylation (BROD). The benefits of the new culturing conditions are improved handling, higher throughput towards full automation and cost efficiency. In addition, high maximal cell densities and an improved viability for all three cell lines were obtained. Furthermore, a high repeatability and process stability due to the chemically-defined, serum-free medium were achieved. The cultivation mode as permanent suspension cell culture facilitates high-throughput and assay flexibility. Thus, the newly established cell lines allow for multiple parallel outputs which can be assessed by single dry or solved sample exposure and subsequent division into different cavities for individual assessment. This new approach with analytical suspension cell lines in a single serum-free, chemically defined medium allows an improved, flexible and sustainable test application as well as a new level of automatization in high-throughput applications.

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In vitro hepatic and gastro intestinal biotransformation data for hydrophobic chemicals in fish: Consideration of gastrointestinal biotransformation in in vitro to in vivo extrapolation models

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An improved understanding of chemical biotransformation in fish has been identified as a critical requirement for the environmental assessment of commercial chemicals. When a chemical is biotransformed, its potential to bioaccumulate in the organism is reduced. Hepatic *in vitro* biotransformation tests, in combination with in vitro-in vivo extrapolation (IVIVE), and bioaccumulation modeling is one initiative to complement regulatory bioaccumulation assessment. While hepatic biotransformation assays are useful in chemical bioaccumulation assessment, the gastrointestinal tract (GIT) may contribute substantially to the elimination of hydrophobic environmental contaminants from fish. The diet is often the primary route of exposure for such compounds, yet the influence of intestinal metabolism on the fate of these chemicals is generally overlooked. Standardized methods for measuring intestinal metabolism in fish and incorporating this information into predictive models for chemical bioaccumulation do not currently exist. In this study we measured *in vitro* biotransformation rates of hydrophobic chemicals (log K_{OW} >4) in rainbow trout hepatic and GIT *in vitro* systems. Selected chemicals included two organic sunscreen agents (ethylhexyl trimethoxycinnamate and octocrylene) and two PAHs (pyrene and benzo(a)pyrene) as the rates obtained in the GIT and liver *in vitro* systems could be compared to previously collected *in vivo* data. Phase I and II enzyme assays were also conducted to compare GIT and liver activities. Additionally, we developed scaling factors to extrapolate *in vitro* activity in GIT S9 to the intact tissue. Fish were dissected to measure the total weight of GIT tissue. Different parts of the GIT tissue were fixed and stained. Using image analysis software we determined the volume of tissue that consists of columnar epithelium, assumed to be the primary site of metabolizing enzymes. We further explored the potential of an updated and expanded IVIVE model that considers metabolism in both tissues.

Mechanistic ecotoxicology of engineered nanomaterials: lessons learnt from human models

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Effectiveness of Dissolution and Bandgap paradigms to predict the toxicity of metal oxide nanoparticles in the marine environment: evidence from ZnO and Mn2O3

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Dissolution and banggap paradigms have been demonstrated to be effective in predicting the ability of metal oxide nanoparticles (NPs) to induce oxidative stress in different *in vitro, in vivo* and bacterial models. The present work addresses the effectiveness of these paradigms *in vivo* and under conditions typical of the marine environment, the final sink of realised NPs. The experiments were carried out by using ZnO and Mn_2O_3 NPs as models for dissolution and bandgap paradigms, respectively. CeO_2 NPs were also considered to assess the potential ability of producing ROS via Fenton like reactions *in vivo*. The hypotheses were tested by exposing for 24 hrs oyster embryos over a wide range of NPs concentrations (i.e. 0.5, 5, 50, 500 μM) and by looking at the oxidative stress as primary toxicity pathway. This work provided evidence about the effectiveness of the dissolution paradigm for predicting NP toxicity in the marine environment. The validity of the bandgap paradigm is also being considered with respect to seawater driven changes to the particles' physical-chemical proprieties. The toxicity assessment was obtained from measurements of oxidative stress signals over three levels of biological complexity and framed into the already established *Hierarchical Oxidative Stress Paradigm*, which describes the strength of oxidative stress as the progressive induction of the cellular antioxidant defence, pro-inflammatory responses and cytotoxicity. The multi-tier toxicological screening system included the expression of selected target genes (RT-qPCR approach), functional responses (Superoxide dismutase activity), pathogenic outcomes (lipids peroxidation) and measurements of whole organism fitness (arrested development of embryos into the first larval stage). An in depth study was carried out to explain the fate and behaviour of Mn_2O_3 under the exposure conditions.Microscopic imaging of the exposed embryo samples was used to assess the effective ingestion of the nanomaterials by embryos. Furthermore a full secondary characterization of the physico-chemical NPs properties in seawater and a set of abiotic screening tests tailored on the bandgap oxidation mechanism were carried out to evaluate changes to the oxidation potential of NPs' surface induced by seawater.

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Releases from transparent blue automobile coatings containing nanoscale copper phthalocyanine and their effects on macrophages

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Cu phthalocyanine is a pigment widely used in paints and coatings. With the advances in materials science, nanoscale copper phthalocyanine (n-CuPc) was added in polymeric dispersing agents to make transparent blue automobile coatings. The use of n-CuPc in the new automobile coatings can potentially lead to its release and induce the potential environmental and occupational risks. Given the increased use of n CuPc in automotive industry, assessing the toxicity of released n-CuPc is important for future n-CuPc applications. This is the first study addressing the cytotoxicity of released n-CuPc from transparent blue automobile toward macrophages. Here we show the matrix played an important role in the release of n-CuPc fragment from automobile coatings and dominated the toxicity of released fragments, preventing the toxicity of the embedded n-CuPc in automobile coatings. Our finding provides a new safety information for automotive industry using nanoscale transparent Cu Phthalocyanine for advanced coatings.

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Challenging sea urchin defense with aminated polystyrene nanoparticles: the evidence of newly acquired particle's biological identity

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The marine environment is likely to be particularly affected by potentially noxious engineered nanoparticles (NPs). Many features peculiar of NPs need to be taken into account when performing nano-ecotoxicological studies, in particular the predisposition to adsorb proteins from the surrounding biological environment, forming the so called "protein corona". We investigated the ecotoxicity of blue fluorescent aminated ($PSNH_2$) NPs to immune cells of the Mediterranean sea urchin *Paracentrotus lividus,* assessing particles' surface modifications as a key aspect of NP ecotoxicity. In order to do so, the coelomic fluid (CF) of adult sea urchin was used for $PSNH_2$ NPs incubation. First, we monitored the dispersion behaviour up to 24 h via dynamic light scattering (DLS), which showed that NPs had a larger average size upon corona formation yet maintaining a remarkably good colloidal stability due to protein steric repulsion. $PSNH_2$ NPs were additionally imaged by transmission electron microscopy (TEM), further confirming the presence of a tightly retained biological coating. The corona was stripped off the NP surface and separated by monodimensional electrophoresis (1D-GE), consequently identifying bands of interest with nano-HPLC-ESI-MS/MS. The protein corona resulted particularly enriched in toposome polypeptide precursor (160 kDa), an iron-less, calcium binding transferrin, which is the subunit of the hexameric toposome protein. Such protein is essential in cell-cell adhesion during sea urchin embryo development and is also associated with stress conditions in sea urchin immune system cells. NP-protein complexes disposition in sea urchin immune cells (coelomocytes) was investigated with optical fluorescence microscopy for 24 hours and $PSNH_2$ NPs were found to be associated only with the phagocytes cellular sub-population, allowing us to hypothesize a selective uptake process. Finally, the effect of $PSNH_2$ NPs on ATP-binding cassette (ABC) transporters was probed with the calcein-AM (C-AM) intracellular accumulation assay that showed a 30% reduction of C-AM internalization in cells co-exposed with NPs (25 μg mL^{-1}), which was eventually prevented when the ABCC1 blocker MK-571 was added. Overall, our data suggest that $PSNH_2$ NPs can acquire a new biological identity upon contact with *P. lividus* CF and that NP-protein complexes may increase the activity of ABC transporters.

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TBD

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Poster spotlight: TU071, TU072, TU073, TU074

Aquatic and Terrestrial Plant State-of-the-art Research linking ecotoxicology and exposure of chemicals

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Effects of the herbicide metsulfuron-methyl on a plant community, including seed germination success in the F1 generation
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A field trial was set up to simulate a field margin environment to analyse sub-lethal effects of the herbicide metsulfuron-methyl on several endpoints of non-target terrestrial plants (NTTPs). For this purpose, seeds of eleven NTTPs were sown in a strip (margin) of 8 m wide and 100 m long. Of the planted seeds 7 terrestrial plant species settled in the margin. The treatments consisted of 1 control and 5 dosages: 0, 0.0097, 0.0193, 0.058 (=HC5 based on vegetative endpoints), 0.174 and 0.348 g a.i./ha. Vegetative and reproductive endpoints were assessed in separate parts of the plots during one growing season. The seeds of 4 species were used in a germination experiment to test the seed germination success of the F1 generation. The herbicide treatments had very little effect on the biomass of the plant species, except for *M. recutita*. Significant differences between the treatments were found for this species ($p< 0.05$, Kruskall Wallis test) with an ED_{50} of 0.06 g a.i./ha and a NOED of 0.0193 g a.i./ha (MMD: 72.02%). For the other species, intraspecific variability was high among the plots. Reproductive endpoints, especially "mass per seed" and "number of seeds per fruit", seemed to be more sensitive than vegetative endpoints. The results of the germination experiment to test whether the herbicide had an impact on the next generation showed that 3 out of 4 species (*S. alba, P. tanacetifolia* and *C. cyanus)* had significantly lower seed germination rates at herbicide dosages of 0.0193 g a.i./ha and higher. The plant species involved reacted differently to the herbicide. *S. alba, C. cyanus and P. tanacetifolia* were highly affected in terms of reproductive endpoints and seed germination, while biomass proved more sensitive in *M. recutita*. Plant reproductive endpoints and germination experiments should be included in this type of risk assessment to investigate the total herbicide effect on the full life cycle and population fitness of the plants.

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Non-Target Terrestrial Plant Risk assessment - background of current proposals based on literature reviews and analyses for SETAC AG Plants
H. Christl, Tier3 Solutions GmbH
Two literature reviews were performed comparing crop and wild plant species and vegetative and reproductive endpoints for NTTPs. Further aspects of the proposed changes in the NTTP risk assessment are presented here, in particular around the effect level considered relevant for the risk assessment, and the use of HR05 throughout. Conclusions are that there is no evidence for intrinsic differences in sensitivity between crop species and wild plant species. Also available data appear not to support a general requirement for testing reproductive endpoints. The proposed change of the effect level (ER10) does not increase reliability, is not regarded to be ecologically relevant, and also cannot be reliably measured as experimental scatter in NTTP test systems is usually greater than 10%; and the use of HR05 may unnecessarily discriminate against selective herbicides compared to less specific products. The desired increased margin of safety could be implemented simply by changing the assessment factor, *if needed*; or by using ER25 endpoints as an alternative. The currently proposed changes would increase bufferzones widths by a factor >10; any filter function of the tier-1-RA (to differentiate normal and problematic cases among active substances and products to be notified) would be lost. *Author on behalf of the SETAC workshops on "Non-target terrestrial plants"

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Monitoring of biomarkers of heavy metals stress during germination of date palm (Phoenix dactylifera L.) seeds exposed to copper treatment
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Copper (Cu) is an essential redox-active transition metal involved in many physiological processes. However, exposure to an excess of Cu has detrimental effects on plant growth which respond in some measurable and predictable manner by implementing mechanisms at different biological organization levels. For instance, Cu may affect gene expression, biochemical activities, cellular and physiological responses. Infra-individual variations of biological responses and selected effector variations can be used as biomarkers to better understand the effects and impacts of an excess of Cu on organisms. Some biomarkers exhibit a good degree of specificity. For example, metalothionein gene expression may vary following exposure to various metals such as cadmium (Cd) and chromium (Cr). Studying gene expression profiles is a good mean to explore the metabolic changes which occur when organisms are exposed to contaminants. Moreover, they are also "early warning" signals that can be potentiallly useful in environmental monitoring. In the present study, we investigate seed germination parameters, oxidative status and gene expression level of genes involved in metal detoxicfication in seeds and seedlings exposed to copper. Seeds from date palm were germinated in direct contact with different Cu solutions (0.02, 0.2 and 2 mM) prepared in distilled water using cupric sulfate ($CuSO_4 . 5H_2O$, Sigma). Hypocotyls were excised from seeds at 12, 15, 30, 45 and 60 days after germination. At low Cu concentration (0.02 mM), only changes in *PdMT* gene expression were significant. Highest cupric ions amount maintained redox balance heavier in their oxidative side and down-regulated the expression of metallothionein coding gene. The maintenance of *ex-situ* date palm seed viability over long periods of copper stress is a key element in monitoring of potential tolerance mechanisms \n

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Growth of Myriophyllum spicatum under semi-field conditions and the resulting key information for the environmental risk assessment of Plant Protection Products
K. Kuhl, Bayer CropScience AG; T. Hall, Bayer CropScience / Environmental Toxicology and Risk Assessment; S. Heine, Bayer Ag / Section IV Environmental Risk Assessment of Pharmaceuticals; A. Solga, Bayer AG Crop Science Division; E. Bruns, Bayer AG, Division Bayer CropScience / Ecotoxicology
In the last two decades the environmental risk assessment for primary producers in the aquatic compartment received an increasing interest. Since March 2006 the OECD guideline 221 is adopted and testing of *Lemna* became a data requirement for herbicides in the EU. At the SETAC EU workshop "AMRAP" (Maltby et al., 2010), which took place in January 2008 in Wageningen, the risk assessment of aquatic marcophytes was discussed.This resulted in an investigation of the protectiveness of the environmental risk assessment for primary producers. The evaluation of SSD's of numerous compounds revealed that in the upmost number of cases the combination of *Lemna* and algae species was sufficient. But this statement is not true for all substances and the development of validated and standardized test methods for the aquatic macrophyte *Myriophyllum* was initiated as a follow up of this workshop. Studies performed with algae and *Lemna* are using mostly water-only systems. While discussing *Myriophyllum* as additional test species the important questions with respect to risk assessment were: If neither algae nor *Lemna* are sensitive to an herbicide, were the correct species tested? In case of

rapidly adsorbing compounds, the bioavailability via the water phase might not represent the environmentally relevant compartment. A spiked sediment test with the rooted aquatic macrophyte *Myriophyllum spicatum* could be more relevant. In addition to these two major questions discussed at the AMRAP workshop, it is important to address additional questions, such as the the typical growth patterns of the species in natural conditions and the "recovery" potential of rooted macrophytes. If the relevant information on these topics is available it can be used to improve already existing mechanistic effect models. Having fully validated effects models provides a tool to examine potential effects of numerous exposure routes and patterns. For this purpose the growth of *Myriophyllum spicatum* was investigated under realistic semi-field conditions in 2015 and 2016. The growth pattern over the entire season and the impact of different sediment composition were observed.

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Linking time-variable exposure to effects - Effect modelling as a tool at different assessment levels.

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Knowledge about expected environmental concentrations is necessary to be able to assess the risks of aquatic macrophytes living in edge-of-field surface water systems caused by application of pesticides on fields nearby. These surface water concentrations are predicted by mathematical modelling approaches that take the specific physicochemical properties of a pesticide as well as the application conditions into account. The standard approach at the European level predicts surface water concentrations of a pesticide for an entire year for several scenarios that are representative for Europe. As a first step, the maximum surface water concentration that is predicted during an entire year is used as reference to assess the risk to aquatic non-target organisms for each of the representative scenarios. However, due to the nature of the different surface water scenarios, the exposure patterns are time variable. Therefore, using the maximum surface water concentration to assess the risk to non-target organisms is a conservative approach. In case a short-term exposure of a pesticide is predicted, such as a peak exposure having a duration of one or two days, the risk to aquatic non-target organisms will be overestimated. In this case the risk assessment can be refined by using time weighted average (TWA) concentrations instead of the maximum. However, recently the general applicability of the TWA approach has been questioned and recommendations were given how to judge whether using the TWA approach is acceptable for specific pesticides. In this contribution a modelling approach of an aquatic plant is used to analyse which properties of a pesticide are decisive for the applicability of the TWA approach. Moreover, alternatives are shown to realistically address time-variable exposure in case the TWA approach is not suitable for a specific pesticide. The uptake and elimination rate as well as the internal concentration-response relationship of a pesticide influence the evaluation whether the TWA approach is suitable for a specific pesticide. Using effect modelling to evaluate entire exposure patterns is an alternative to address time-variable exposure in case the TWA approach cannot be applied. Modelling approaches offer several advantages such as the possibility to assess numerous complex exposure patterns that cannot be addressed by toxicological tests due to difficulties mimicking the exposure patterns and the time and labour such tests would require.

Combined effects of chemical and environmental stressors: from local stressors towards climate change (IV)

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Changes in pCO2 alter the uptake and effects of common chemical pollutants in marine worms

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Release of anthropogenic carbon dioxide in to the atmosphere and subsequent absorption by surface waters is resulting in increased pCO_2 in aquatic environments. Furthermore, active pharmaceutical ingredients (APIs) are increasingly being found in sewage effluents and receiving waters, primarily as a result of patient use. Traditionally, these have been studied in isolation, although factors such as pH and pCO_2 can alter ionisation and bioaccumulation potentials of many APIs, altering their toxicity.This study aims to investigate the hypothesis that changes in pCO_2 alter the uptake and effects of common chemical contaminants. We used the ragworm *Hediste diversicolor* to explore this relationship due to it being a dominant estuarine species in contaminated areas, playing major roles in bioturbation and nutrient cycling. A range of APIs were chosen including fluoxetine, cocaine, and neonicotinoid pesticides due to them having chemical properties which may make them susceptible to changes in pCO_2 and having drug targets that are conserved in marine invertebrates. Exposures were performed under pCO_2 conditions equating to pH 8.10, 7.75, 7.40 (± 0.1), representing current and future predictions, and under a range of concentrations (ng to μg L^{-1}) of the selected compounds, spanning environmental relevance. For each, a kinetic bioconcentration factor (BCF) was calculated. Endpoints were measured based around the mode-of-action (MoA) for the compound, including serotonin quantification for fluoxetine and cocaine, and acetylcholinesterase activity for neonicotinoid pesticides. Related endpoints were measured for each, to include: feeding rate, metabolism and predator avoidance behaviour. Our findings demonstrate novel evidence that uptake and effects of common chemical pollutants are affected by pCO_2, including changes in bioconcentration factor and altered MoA-associated measures. Our results indicate that combined stressors can interact in complex ways, which vary within and between chemical groups. As a consequence of population growth and better healthcare provision, chemical pollution of APIs will likely increase. Here, we show that laboratory-exposure studies to determine risk may not adequately characterise potential effects of a compound if only conducted under current environmental parameters. We believe it necessary to future-proof risk assessment by considering how predicted pCO_2 conditions may impact the toxicity of contaminants, in particular APIs.

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Ocean Acidification Responses Lead to Altered Toxicities of Chronic Coastal Pollutants in Marine Invertebrates.

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Ocean acidification (OA) is not happening in isolation but occurring against a background of chronic low-level contamination for many coastal marine environments, yet the potential for OA interactions with coastal contaminants has received very little attention to date. Metals are some of the most wide-spread environmental contaminants and OA is expected to alter their bioavailability to biota by influencing their speciation in seawater,effectively increasing the free ion concentration under higher pCO_2 conditions. We provide evidence from a range of marine invertebrates and life history stages that the sub-lethal toxicity effects of the metal contaminant copper (Cu) are significantly altered when organisms are exposed under near-future pCO_2 levels. For example, in the mussel *Mytilus edulis* copper-induced DNA damage was 90% greater when exposed under OA conditions (1373 µatm pCO_2) compared to ambient conditions (436.4 µatm pCO_2). A similar response was observed in the sea urchin *Psammechinus miliaris*, however the increase in DNA damage was only 23% higher under OA than ambient conditions for this species. Surprisingly, in the shore crab *Carcinus maenas* the level of DNA damage induced by copper exposure decreased by 40% when exposed under OA conditions. We identified a tentative relationship between an organisms ability to acid base regulate in response to OA and the subsequent level of DNA damage induced by exposure to copper. Our results demonstrate that the changes in toxicity observed when copper is exposed under OA conditions are complex, varying between species and life-history stages and hence are not driven purely by changes in chemical behaviour driven by reduced seawater pH. The physiological responses of the organisms to OA, such as their acid-base regulation capacity and changes in ventilation behaviour, appear to play a key role in driving these toxicity responses. Since coastal pollution is widespread the interactions we have demonstrated between an organisms' response to OA and its response to contaminants have significant implications for current predictions of OA impacts on a wide range of coastal marine invertebrates.

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Species-specific vulnerability of Arctic copepods to oil contamination and global warming

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Arctic ecosystems are predicted to have more severe effects from global warming as during the last decades the temperatures have increased in this region at a rate of 2-4 times higher than the global average. In addition, oil exploitation and shipping activities in the Arctic are predicted to increase under global warming as the result of the retreat of sea ice, posing the risk of oil contamination. It is poorly known how cold adapted copepods in the Arctic deal with the combined effects of global warming and oil exposure. To address this, we exposed females of two copepods species *Calanus glacialis* and *C. finmarchicus* to pyrene at three temperatures: 2, 6 and 10°C. Both species co-exist in the Disko Bay, Greenland, but only *C. glacialis* is a true Arctic species while *C. finmarchicus* is of north Atlantic origin. Pyrene is one of the most toxic components of crude oil to marine copepods. The temperatures of 2, 6 and 10°C represent the mean sea water temperature during the reproductive season, the 4°C increase in mean temperature by 2100 as predicted by IPCC scenario RCP8.5 (2013) and the extreme sea water temperature, respectively, in Disko Bay. Four-degree temperature increase did not have an effect on grazing rate and survival of both species. However, the extreme temperature (10°C) increased the grazing rate and mortality of *C. glacialis*, but not in *C. finmarchicus*. Exposure to high pyrene strongly reduced survival and grazing rate in both species and this pattern was independent of temperatures. Notably, exposure to high pyrene resulted in ca. 70% of mortality in *C. finmarchicus*, the species with North Atlantic Origin, that was two times higher than the mortality observed for *C. glacialis*, the true Arctic species. These results suggest that extreme temperature under global warming and oil contamination may drastically change the relative abundance of the Arctic pelagic copepod community by changing the species-specific vulnerability to extreme temperature and oil exposure.

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Additive Effects of Multiple Pharmaceuticals on Tadpole Responses to a Natural Stressor
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Animals face many natural stresses in their daily life and increasingly face multiple stressors of anthropogenic origin. The presence of predators in the immediate environment can be a major source of stress. Tadpoles can detect diffusible cues (kairomones) from predatory dragonfly larvae, and normally respond by reducing their swimming activity. The pharmaceuticals fluoxetine, sertraline and venlafaxine are widely prescribed for the treatment of depression and anxiety. These drugs belong to a group of chemicals that selectively inhibit the reuptake of serotonin or norepinephrine in the brain. All three drugs have been detected as contaminants in surface waters, downstream of waste treatment plants. A number of studies have demonstrated that these drugs can alter the behaviour of wildlife, however, little is known of their combined effects. Tadpoles of the Arabian toad (*Duttaphrynus arabicus*) were exposed for 14 days to either 0.5 or 2.0 μg/L of either fluoxetine, sertraline and venlafaxine alone or a combination of all three at their low or high concentrations. After exposure, the tadpoles were individually transferred to glass Petri dishes filled with freshwater containing freshly prepared dragonfly kairomones. After a 15 minutes acclimatisation period, swimming behaviour was recorded for 30 minutes at 1.9 frames/sec. The video was subsequently analysed using the program CTRAX. There was no effect of any treatment on the survival or mass of the tadpoles (P>0.05). The average swimming speed of the controls was 9.1±3.4 cm/s. Exposure to fluoxetine, sertraline or venlafaxine individually at 0.5 μg/L significantly increased average swimming speed to 29.1±2.1, 16.8±3.3, or 28.96.7 cm/s respectively. In contrast, exposure to 2.0 μg/L of the drugs alone, or a combination of the three drugs each at 0.5 or 2.0 μg/L had no effect on swimming speed compared with the controls. Increased swimming activity may increase the risk of encounter with a predator and is likely a maladaptive response. The effects of the three drugs alone (effectively 1.5 μg/L total dose) was similar to a single drug at 2.0 μg/L. These results indicate that the effects of the three drugs on the behavioural responses of *D. arabicus* tadpoles to predator kairomones are dose-dependent, but follow an additive mode of action.

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Effect of the succession of endosulfan exposure and bacterial challenge on DNA integrity and non specific immunity of the three-spine stickleback, Gasterosteus aculeatus.
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Endosulfan ($C_9H_6Cl_6O_3S$) is an active substance of phytosanitary products widely found in surface water, groundwater, sediment and rain of many regions in the world and an important source of poisoning in many countries. Endosulfan is known to have genotoxic and immunostimulating effects such as increase of phagocytic capacity. The immune system, in particular innate immune response, seems to be very attractive for biomonitoring due to its connection with organism health status. Indeed, fish weakened by chemical stress might be unable to resist against pathogen aggression. In fact, a connection has been established between the destabilization of the fish immune system, caused by sub-lethal doses of contaminants, and a defect in fish pathogen resistance. In this context, the stresses induced by chemical (chlorpyrifos) and succession of chemical/pathogens (LPS endotoxin) were determined in the three-spined stickleback, *Gasterosteus aculeatus*, after 4 days of exposure. A loss of DNA integrity was observed after exposure to 1.75 μg.L during 4 days. An increase of splenic leucocytes phagocytic abilities was observed in addition of a reduction of oxidative stress. Moreover, the injection of LPS following endosulfan exposure seems to provoke a destabilization of fish immune system with drastic decrease of phagocytic efficiency and increase of cell mortality by apoptosis. These results may be linked to endocrine disruptor properties of endosulfan and its ability to mimic cytokine signal. This study points out the importance of stress on stress experiments to determine all potential effects of chemical exposure in fish.

Insights and challenges concerning the bioavailability of organic chemicals and communication implementation in risk assessment

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Bioaccessibility of native PAH and derivatives in a fuel soot using an in vitro GI model
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Fossil fuel soot particles are ubiquitous in soil and on indoor and outdoor environmental surfaces and therefore can be a significant source of entrained contaminants through hand-to-mouth activities, especially in children. We determined the apparent bioaccessibility (B_{app}) of eleven PAHs, one nitro-PAH, and four oxo-PAH derivatives natively present in a fuel oil soot from commercial boilers using an *in vitro* three-stage gastrointestinal model. We also calculated a limiting bioaccessibility (B_{lim}). The model included silicone polymer sheet as an absorptive sink in the small intestinal stage to mimic uptake by the small intestinal epithelium. The soot-borne contaminants were regarded to exist in either a labile or a nonlabile state. Silicone absorption was expected to promote desorption by steepening the concentration gradient across the soot-fluid interface. The B_{app} and B_{lim} included the sum of fluid-phase and silicone-phase chemical. As predicted, silicone significantly increased B_{app} by reducing the labile fraction remaining on the soot residue after digestion. The B_{app} was independent of gastric pH and added dietary proteins and carbohydrates, but increased with bile acids concentration, small intestinal pH (5.00–7.35), and added dietary lipids. Rising small intestinal pH favored mass transfer from nonlabile to labile states, from labile states to digestive fluid, and from digestive fluid to silicone. The addition of lipids favored mass transfer from nonlabile to labile states, and from labile states to bile acid/lipid mixed micelles. The extrapolated B_{lim} ranged from ~30 to ~65% among the PAHs and PAH derivatives, which were surprisingly high. The B_{lim} was not correlated with molecular size or K_{ow}, suggesting that a major determinant of bioaccessibility is not contaminant hydrophobicity, but the original distribution of the contaminant between nonlabile and labile states, as dictated by the path of its introduction into/on the soot. The results indicate significant variability in soot PAH bioaccessibility within the range of physiological conditions experienced by humans, and suggest that bioaccessibility will increase with co-consumption of food, especially food high in fat.

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Application of an antibody-based biosensor for rapid assessment of PAH fate and toxicity at contaminated sediment sites
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Hydrophobic contaminants such as polycyclic aromatic hydrocarbons (PAH) accumulate in sediments and may pose significant ecological and human health risk. Contaminant bioavailability is governed by partitioning but multiple chemical and physical factors influence bioavailability in natural systems. Because measuring contaminant exposure and uptake in biota is time consuming and expensive, multi-phase models have been developed to predict contaminant bioavailability. The heterogeneity of natural habitats makes it difficult to reliably predict bioavailability from measured bulk sediment concentrations and properties. Ultimately, site-specific pore water measurements are needed to accurately predict contaminant fate and bioavailability but these measurements are difficult to obtain by standard analytical methods. Advances in biosensor technology allows near real-time measurement of contaminants at sub ppb concentrations in small volume (< 5 mL) aqueous samples. A quantitative, monoclonal antibody (mAb)-based sensor was used to measure PAH concentrations in sediment pore water collected at various PAH contaminated sites in the Elizabeth River, VA and Baltimore Harbor, MD. PAH concentrations in pore water samples were measured in the field within minutes of collection with the biosensor to map the spatial distribution of PAHs at contaminated sites in the Elizabeth River, VA. Sampling of pore water concentrations with depth by drive-point piezometer allowed vertical profiling of PAH concentrations within sediments to evaluate the input of contaminated groundwater and document mixing of surface water within the sediments. Analysis of PAH concentrations in pore waters from sediment cores collected in Baltimore Harbor was used to evaluate PAH toxicity to benthic amphipods. Correlations between PAH concentrations measured by the biosensor (< 1 μg/L to > 600 μg/L) and those measured by GC-MS were excellent and the results are being used to help develop sediment remediation strategies.

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Bioavailability concentration of PAHs quantified with passive samplers in the same soil under ex situ, semi in situ and in situ conditions
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Large success has been achieved in applying *in situ* passive sampling (PS) techniques for sensing hydrophobic organic contaminants such as polycyclic aromatic hydrocarbons (PAHs) in environmental compartments such as air, water and sediments. However, there is very little information on the capability of these methods to assess the bioavailability of PAHs in soil. In standard *ex situ* methods soil samples are dried, sieved and then brought in an aqueous suspension into contact with a PS. In contrast, *in situ* PS methods have the advantage of taking automatically and integratively into account actual environmental conditions such

as temperature, ionic strength and soil water content. Here, we compared the results of a traditional *ex situ* method with those obtained directly under *in situ* field conditions. To bridge the gap between the two, a semi *in situ* pot experiment was also done. Here, we kept some pots at unsaturated water conditions and others at full saturation. The three experiments were carried out with a contaminated soil with 270 mg/kg summed total concentration of the 16 US EPA PAHs. All the experiments were carried out with two different PS methods. The first one is low density polyethylene (LDPE) [1]. Performance reference compounds (PRCs) were spiked in the LDPE before starting the experiment to assess whether equilibrium was reached. The second PS was polydimethylsiloxane (PDMS) glass fibers [2]. Fibers of two different thicknesses were used to provide the information whether equilibrium was reached. The objective of this study is to investigate the concordance of the PS results, from the different experiments to know what information is provided by *in situ* PS bioavailability analysis. Preliminary LDPE results showed that after nine months PRCs desorbed over time in accordance with their hydrophobicity. The PRCs results helped to estimate that in the semi *in situ* experiment, PAHs with a $\log_{10}K_{ow}< 5.5$ have already reached equilibirum. For the fully *in situ* situation only PAHs with $\log_{10}K_{ow}< 4.5$ have equilibrated. To our knowledge, this is the first experiment where PS were deployed directly in the soil and exposed to environmental conditions. The outcome will help to evaluate whether *in situ* PS is a tool to be eventually included in risk assessment and legislation References: 1. Booij, K., F. Smedes, and E.M. van Weerlee. Chemosphere, 2002. **46**(8): p. 1157-1161. 2. Witt, G., et al. Environmental science & technology, 2013. **47**(14): p. 7830-7839.

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How to consider bioavailability in the assessment of construction products

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Construction products are complex materials and contain a large number of different chemicals, which might be released to the environment during usage. Depending on the compound and on the matrix only a small portion is transferred to environment and therefore a risk assessment should be based on the available portion. Concerning the pathway to soil and groundwater by contact with rain or seepage water leaching tests were developed and standardized by CEN to determine the release potential of construction products. Chemical analysis in the eluates can be combined with eco-toxicological testing. In this presentation an overview about existing leaching methods is given using selected examples of our work. We especially draw the attention to research which is still needed to close the gap between the results of leaching experiments and the subsequent risk assessment of the products. The eluates of 15 paints for outdoor use were used for a screening of organic compounds and the concentration of cations and anions were determined. Concerning the inorganic constituents only a small number of elements were present with concentrations higher than the limit of detection. Thus, leaching of potential hazardous chemicals is probably of higher concern. Several glycol ethers, polyalkylene glycols and some surfactants and coalescing agents have been identified. Among these only the latter are classified as hazardous to aquatic organisms. The identification of the organic compounds is a time consuming and labor-intensive step due to the high number of chemicals produced and used. Using eco-toxicity tests provides information on the environmental risk without the need for of chemical identification of all compounds. The screening of a set of construction products shows that only a part of eluates causes adverse effects in the test and that such screening can be used as a first step in the assessment of construction products. If this kind of testing is included in the evaluation concept an in depth chemical analysis is only needed for products showing adverse effects. Determination of the eluate concentration after leaching enables to determine the potential release to the environment and to focus on the available fraction. However, limit values or other concepts to use this information in risk assessment are still missing.

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Advances in the Development of Procedures to Establish the Toxicity of Non-Extractable Residues (NER) in soil

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There is already a long discussion around the bioavailability and ecotoxicological relevance of Non Extractable Residues (NER) in soil. Is NER formation a detoxification process or should it be considered to be a hidden hazard? The formation and presence of NER may be attributable to: 1) Association of the parent chemical or breakdown product with mineral and/or organic matter, 2) Mineralisation and incorporation of carbon into microbial biomass and carbonates. NER can only be established using labelled compounds (e.g. ^{14}C) and cannot be measured with conventional chemical analytics. Regulations ask for understandable and measurable parameters. The approach of Ortega-Calvo et al. (2015) has been followed and considered are: The water phase, in which concentrations/activity can be measured using passive sampling or extractions with 0.01 $CaCl_2$ (Actual availability); A potentially available fraction in equilibrium with the water phase, measured using a Tenax extraction (ISO/ DIS 16751); The total extractable amount, measured with a (standard) method designed for the total substance amount; NER is considered, but mentioned as non-measurable and also non-bioavailable. All other fractions are measurable. In addition, the distribution of the chemical over the above fractions and the residual amount (still present after removing of the total extractable amount) was measured using ^{14}C-labelled compounds. With ^{14}C-experiments radio activity is measured but the substance identity remains unknown. The distribution in the soil of ^{14}C was followed for 6 months. Toxicity was tested in both freshly spiked and aged soils. TNT, Cypermethrin and Carbendazim were used as test chemicals and results will be presented. For ^{14}C-labelled TNT for example, it was shown, that 6 months after spiking most of TNT is present as NER. The NER formation caused a large reduction of toxicity in earthworm avoidance tests and *Vibrio Fischeri* luminescence. Removal of the potentially available fraction, using TENAX, also removed the toxicity in freshly spiked and aged soil. This potentially available fraction therefore explains the toxicity. In spite of the impossibility to measure NER directly in real environmental samples, it will be shown that the methods developed provide reliable indirect information that can be used to assess behaviour and toxicity of substances that are known to form Non Extractable Residues. The approach is logic and based on measurable parameters.

Antibiotics and Antibiotic Resistance in the Environment: Ecological Fate and Effects, Resistance Development and Implications for Human Health

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Analytical tools for the holistic assesment of antimicrobial distribution and associated risks in aquatic ecosystems

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Nowadays there is an increasing concern about the development of antibiotic resistant bacteria in the environment and its implications for human health. After administration, antibiotics and their metabolites are released into the water streams and reach wastewater treatment plants (WWTPs), where they are not efficiently removed, being therefore continuously discharged into the environment. Also, veterinary antibiotics used in animal farms and aquaculture, may be released together with animal wastes through overflow or leakage from land application or storage structures [1,2]. Past work has shown that the presence of certain levels of antibiotics in river waters may promote the development of antibiotic-resistant genes in the local bacteria [3]. Few studies have shown that even at the very low antibiotic concentrations measured in some aquatic and soil environments, maintenance and selection of resistant bacteria can occur. The minimum chemical concentration required to provide a selective advantage to the microorganisms carrying the resistance gene, relative to the same bacterium that is sensitive to the chemical, it is called minimum selective concentration (MSC). The objective of the present work was to provide a series of analytical tools (validated quantitative and sensitive analytical methods) to assess the ambiental concentrations of selected fluoroquinolones, quinolones, macrolides, penicillins, sulfonamides and their metabolites, and tetraciclines, in the different environmental compartments including surface water, groundwater, marine coastal water, sediment and biota. The data obtained allowed to associate their occurrence with the potential environmental risk posed. References [1] Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ Sci Technol 36:1202-1211. [2] Berkner S, Konradi S, Schönfeld J. 2014. Antibiotic resistance and the environment—there and back again. EMBO rep 15:740-744 [3] Martínez JL. 2008. Antibiotics and Antibiotic Resistance Genes in Natural Environments. *Science* 321(5887):365-367. ***Acknowledgements -*** This work was funded by the EU through the Project Sea on a Chip (Contract 614168) and by the Generalitat de Catalunya (Consolidated Research Group “2014 SGR 418 – Water and Soil Quality Unit”).

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Low-energy Downflow Hanging Sponge (DHS) bioreactors reduce antibiotic resistance genes for decentralised wastewater traetment applications

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Billions of people worldwide do not have adequate domestic wastewater treatment, which leads to the spread of infectious disease and antimicrobial resistance (AMR) via contaminated water. This results in an estimated 2.1 million deaths every year and this is particularly evident in the emerging world, where central sewage collection is not economical and limited effective small-scale treatment options exist in the expanding peri-urban communities. Recent work has shown Downflow Hanging Sponge (DHS) bioreactors have potential for decentralised waste treatment. DHS reactors include an upper sponge layer that has passive aeration and a lower sponge layer, submerged by effluent from the upper aerobic layer. Our DHS design includes a wastewater bypass that provides additional carbon to the lower layer to ensure anoxic conditions, creating sequential aerobic-anoxic conditions. DHS systems removed >85% and >74% chemical oxygen demand (COD) and total nitrogen (TN), respectively, and used limited energy during operations. To assess how the systems impacted antibiotic resistance gene (ARG) and mobile genetic element (MGE) levels in wastewater, a series of DHS reactors were operated using different percent bypass ratios, and influent and effluent resistomes were quantified. All configurations reduced ARG and MGE levels by over 60%, although optimal removals (>80%) were seen at 20% bypass ratios. Aminoglycoside, tetracycline, and β-lactam ARGs were removed most efficiently, although slightly elevated effluent MGE levels were seen at higher bypass ratios. Overall, DHS reactors are very promising for inexpensively reducing ARG loads in domestic wastewater and should be promoted for desentralised use in the emerging world.

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How can we improve the environmental assessment for antibiotics? Considerations for species sensitivity, antimicrobial resistance and ecosystem function
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Antibiotics are vital in the treatment of infectious disease in both livestock and human health and enter the environment continuously. In freshwaters antibiotics can reach concentrations up to mg/L, but more commonly they occur in the low ug/L range. They selectively target bacteria and thus there is a high likelihood for adverse effects on environmental bacteria populations at levels well below that for effects on aquatic vertebrates. Current environmental risk assessment (ERA) of antibiotics, as required by the European Medicines Agency guidelines 2006, adopts the use of one species of cyanobacteria to represent all bacterial diversity, and an activated sludge respiration inhibition test (ASRIT) that is insensitive for antibiotics, is used to identify risk to microorganisms in sewerage treatment plants. Thus there is concern that the ERA for antibiotics does not fully consider their potential impacts on microbial community structure, function and resilience. Adding to this, it has been proposed that the risk of antimicrobial resistance (AMR) development in the natural environment should be included in ERA, but there is currently no standard experimental methodology. To address this, recently, a method for the theoretical determination of a predicted no effect concentration (PNEC) for AMR has been proposed by Bengtsson-Palme and Larsson (2016) that uses minimum inhibitory concentrations from clinically relevant bacteria. We conducted a meta-analysis on all available data for taxa commonly used in ecotoxicity testing. We use the findings to help define science-based protection goals for antibiotics for use in a prospective regulatory frameworks for ERA and to define safe discharge concentrations for antibiotic production, taking into consideration both population based traditional aquatic ecotoxicty endpoints and AMR development*,* Our key finding are: 1) the sensitivity range for cyanobacteria extends over two orders of magnitude between species and thus a more diverse range of bacteria species may be warranted to protect bacteria communities adequately and the ecosystem functions they provide; 2) metazoan species are several orders of magnitude less sensitive to antibiotics than bacteria, as expected, and this may question the need for their inclusion in the ERA; 3) a predicted no effect concentration (PNEC) for AMR would not always be protective of the current PNEC, highlighting the importance of considering both in protection goals.

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Simple assay for determining MSCs of antibiotics
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Antimicrobial resistance is one of the most significant threats to global public health and economy in the modern era[1]. Use, misuse and overuse of antibiotics in humans in the clinic and community; and in animals in agriculture and aquaculture results in flow of antibiotics and antibiotic resistant organisms into the natural environment[2]. Though environmental concentrations of antibiotics are very low (mostly in the ng/L range[3]), recent studies have shown that these may exert enough selective pressure to select for antibiotic resistance[4,5,6]. Currently there are no standardised ecotoxicological assays which assess a compound's potential to select for antibiotic resistance. The methods used to determine minimal selective concentrations ('MSCs' - the lowest antibiotic concentration which selects for resistance[4]) in other studies[4,5,6] while effective, can be arduous, expensive and in some cases are based only on single species; meaning they are not representative of the complex communities of bacteria which exist in the natural environment. This work builds on previous studies to determine MSCs in natural complex communities through quantification of resistance genes; and to validate a short, simple, reproducible assay which may be used to determine MSCs rapidly. Results so far suggest selection may occur for some antibiotics at environmentally revelant concentrations, and that growth of a natural complex community may be useful in determining MSCs. [1]O'Neill, 2014. O'Neil Report Wellcome Trust. Review on Antimicrobial Resistance. *Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations* [2]Andersson & Hughes, 2012. Evolution of antibiotic resistance at non-lethal drug concentrations. *Drug Resistance Updates,* 15**,** 162-1 [3]Homem & Santos, 2011. Degradation and removal methods of antibiotics from aqueous matrices - A review. *Journal of Environmental Management,* 92**,** 2304-2347 [4]Gullberg, Albrecht, Karlsson, Sandegren, Andersson, 2014 Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals. *MBio,* 5 [5]Gullberg, Cao, Berg, Ilback, Sandegren, Hughers, Andersson, 2011. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. *Plos Pathogens,* 7 [6]Lundstron, Ostman, Bengtsson-Palme, Rutgersson, Thoudal, Sircar, Blanck, Eriksson, Tsylind, Flach, Larsson,. 2016. Minimal selective concentrations of tetracycline in complex aquatic bacterial biofilms. *Sci Total Environ,* 553**,** 587-595

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Poster spotlight: TU141, TU142, TU143, TU144

Interpreting Biological Effects of Metals and Their Mixtures in the Aquatic and Terrestrial Environment (I)

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Metal bioaccumulation assessment in macroinvertebrates and relationships to benthic community quality state in a mining region
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Bioaccumulation data of As, Cu, Hg, Ni and Se in 10 different macroinvertebrate taxa were used to establish baseline metal values, covering different functional feeding styles. These were calculated for each taxa as the 90th percentile (P90) value of the metal data distribution at 14 reference sites in the Nalón River basin (Asturias, North Spain). Additionally, 16 test sites were studied where the main anthropological pressure was identified as related to Cu, Hg or Au mining activities. The objectives of the study were, first, to assess metal bioaccumulation in selected benthic taxa within a Reference Condition Approach; and second, to assess the relationships of the response of the macroinvertebrate community on metal tissue levels. The aim of this study is to contribute to the database necessary for a future proposal on preventive threshold on metal tissue concentrations in order to protect the benthic macroinvertebrate communities. Seven metrics (METI, NORTI, No. Famililies EPT and PT, Abundance of EPT, PT and Elmidae) were identified to have significant reductions at Medium and High metal bioaccumulation categories for at least 7 out of 10 taxa. Metal bioaccumulation assessment based in the ΣP90-Q was in accordance with the biological EQR evaluation at 75% of the cases, which suggest that the approach is a valuable monitoring tool for bioaccumulation risk assessment and protection plans of freshwater benthic communities. There is not a particular taxon that can be used as a single bioindicator of the metal bioaccumulation risk, either due to its absence in some test sites, or to bioaccumulation pattern variability from metal to metal. Multivariate analysis shows that macroinvertebrate community alterations were best explained by the bioaccumulation of As, Cu and Hg.

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Combined effects of four antifouling compounds on two marine phytoplankton species
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Antifouling paints are used to prevent attachment of organisms to the ship hull. They usually associate copper with biocides like diuron, irgarol, or zinc pyrithione (ZnPT). Leaching of these compounds into the environment induce a contamination of sediments and water in marinas and harbour areas worldwide. Active ingredients in antifouling paints exhibit a high toxicity for various organisms, but the way their toxicity can be modified by interacting together is not well described. Phytoplankton play a key role in the oceanic food web and species living in marinas and harbour areas are exposed to a cocktail of these compounds. Thus, this study aimed to assess the toxicity of binary mixtures of antifouling active ingredients towards the growth of two marine microalgae, *Tisochrysis lutea* (haptophyte) and *Skeletonema marinoi* (diatom). The toxicity tests consisted of a 96-h exposures of phytoplankton to contaminants in sterile 48-well microplates incubated under controlled conditions. Each species was exposed to each substance at 6

concentrations (3 to 6 replicates per treatment). The binary mixture exposures were achieved following a fixed-ratio ray design with 5 mixture ratios (100:0%, 75:25%, 50:50%, 25:75% and 0:100%) for each mixture. The toxicity was assessed by calculating EC_{50} values based on the growth inhibition after 96 h for each compound/mixture. For the analysis of binary mixture results, both Concentration Addition (CA) and Independent Action (IA) reference models were tested against more complex models allowing for synergy and antagony. Single exposure results on *Tisochrysis lutea* showed a very high toxicity of irgarol, diuron and ZnPTwith EC_{50} values below or in the $\mu g\ L^{-1}$ range, while copper was almost reaching the mg L^{-1} range. The toxicity of these 4 compounds can be ranked as follows: irgarol > ZnPT > diuron > copper. The binary mixture results showed various interactions, from additivity following CA predictions for the diuron:irgarol mixture, to significant and strong antagonism for mixtures of diuron and irgarol with copper. Repeating experiments for some mixtures, including more mixture ratios, would be needed to conclude on the interaction. Testing antifouling compounds on two phytoplankton species from different phyla should allow to statuate whether or not a general interaction pattern can be highlighted with such mixtures on microalgae. It could then be of interest to seek for the mechanisms causing antagonistic interactions.

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Sensitivity of Caenorhabditis elegans to mixtures of zinc, copper and cadmium

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Industrial and natural sources of heavy metals cause an increase in metal accumulation, which can lead to serious health hazards for diverse animals including humans, resulting in a persistent (eco)toxicological concern. In contrast to the toxic effects of individual metals, very little is known about their interactions and putative additive effects in the environment. Especially soils, sediments and surface waters can be contaminated with metal mixtures. Since soil nematodes live within the interstitial waters of soil particles, they are in direct contact with dissolved contaminants and are thus good models for these toxicity tests. One of the major challenges in ecotoxicology is to obtain insights in mixture toxicology to set realistic environmental quality criteria. Therefore, the aim of this study was to gain insights into the sensitivity to the selected metals (Cu, Cd, Zn), and to investigate whether and how these sensitivities are affected in mixture exposure scenarios, To assess different endpoints, we fully exploited the benefits of the free-living soil nematode *Caenorhabditis elegans* as a unique model to investigate the effects of metal exposure on survival rate and behavioural responses. *C. elegans* exhibited a different sensitivity to the three metals, both as individual metals as in combination. Different interaction effects were observed for the mixtures ZnCd, ZnCu, CuCd and ZnCuCd. Our study shows that even at low concentrations the locomotion, both on agar plates and in liquid medium, was disturbed.

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Effect of temperature on chronic Ni toxicity to Daphnia magna along four generations

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Ecological risk assessment (ERA) is based on single generation ecotoxicological tests that are usually performed at a standard temperature. A previous in-house study indicated that temperature had a significant effect on chronic nickel (Ni) toxicity *to Daphnia magna.* Previous multigenerational studies performed at one standard temperature (20°C) showed that Ni toxicity increases along generations. Therefore, our goal was to investigate the effect of temperature on chronic Ni toxicity to *D. magna* along four generations. A multigenerational toxicity test was performed with *D. magna* exposed to Ni along four generations at 15, 20 and 25°C. Prior to actual Ni exposure, the organisms were acclimated during two generations to the temperature treatments. At the time of exposure, each generation started with neonates (< 24h) collected from the 3rd brood of at least three different mothers. Each generation continued until the organisms in control treatments released the 5th brood. Corroborating the previous in-house study our results showed that temperature had a significant effect on chronic Ni toxicity to *D. magna* in the 1st generation. The estimated 10% effect concentration on reproduction per individual female untill the 5th brood (EC_{10}) for Ni increased 20-fold between 15 and 25ºC on F0. We observed that when *D. magna* was exposed to Ni treatments ≤ EC_{10}s (calculated for F0) chronic Ni toxicity did not increase along generations. This was common to the three temperature treatments. For Ni treatments ≥ EC_{10}s (calculated for F0) chronic Ni toxicity varied along generations but a consistent trend was not observed. At relevant concentrations for ERA (i.e. EC_{10}) chronic Ni toxicity at 15, 20 and 25°C did not increase along generations. However, for F0 the EC_{10} increased 21 fold with the increase of temperature from 15 to 25°C which indicates the need to integrate temperature as a factor in metals risk assessment.

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Does prior exposure to heavy metals protect future generations of plants to metal stress?

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Many anthropogenic activities have contributed to a release of pollutants, including heavy metals, into the environment. As plants are sessile organisms, they cannot avoid exposure to harmful conditions. It is therefore essential for plants that they are able to defend themselves by responding and adapting to harmful environments. The hypothesis of this study is that metal exposure in a previous generation protects *Arabidopsis thaliana* plants against uranium (U)- and cadmium (Cd)-induced stress. Therefore, two different seed-types were used: control seeds (never exposed before to heavy metal stress) and U-seed (exposed in their previous generation to 5 μM U). Plants of both seed types were grown for 18 days after which they were exposed to 5 or 10 μM Cd or to 25 or 50 μM U during 3 days. Effects were generally more pronounced in the roots than in the leaves. This can probably be explained by the low Cd and U concentrations that were translocated to the leaves, suggesting that effects are mainly induced by the direct presence of the metals in the tissues instead of induction through root-to-shoot signalling. In the roots of both seed-types, growth was severely reduced after U and Cd exposure. In addition, differences were observed for different enzymes of the antioxidative defence system and for the concentration of oxidized and reduced glutathione for the different metal treatments but not between the different seed-types. However, at gene expression level, differences between control seeds and U-seeds were observed. As such, a significant increase in the transcript levels of different DNA-repair related genes after metal exposure was observed in the roots for U-seeds as compared to the control seeds, indicating an increased capacity to repair DNA damage. In addition, differences in transcript levels of genes related to DNA methylation were observed, with an increased expression in *MET1*, *DRM2* and *CMT3* in the U-seeds as compared to the control seeds. Those changes can induce differences in the DNA methylation state of the U-plants which in turn can influence differences in gene expression. In conclusion, while only minor differences were observed between control seeds and U-seeds for the growth responses, antioxidative enzyme capacities and glutathione concentrations; the increased expression of genes involved in DNA repair and genes involved in DNA methylation provide some evidence for a protective ability of metal exposure in a previous generation.

New developments in ecotoxicology for the risk assessment of single and multiple stressors in insect pollinators: from the laboratory to the real world

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Chronic and mixture toxicity of pesticides and environmental contaminants for solitary bees, honeybee and bumblebees : differential sensitivity and mixture interactions?

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For laboratory testing, assessments involving bees generally start with acute studies in the honeybee, *Apis mellifera*. Further, only limited information on mixture effects on bees is available. We have developed and trialled toxicity tests that allow for long-term exposure (up to 240 hours) for three bee species: the honeybee *A. mellifera*, bumblebee *Bombus terrestris* and a solitary bee *Osmia bicornis*. We used a number of different insecticides (clothianidin, dimethoate, tau-fluvalinate), other pesticides (propiconazole, 2,4-D) and environmental contaminants (cadmium and arsenic), as well as selected mixtures. Survival data over time following exposure to all single chemicals and the selected mixtures in the three species were derive and used to fit dynamic energy budget toxicity (DEBtox) models. Results from the short-term tests were found to be between 3 and 25 fold lower than expected from life-time exposure. The response profiles of species over different exposure concentrations and with time were found to be broadly similar indicating similar toxicokinetic, toxicodynamics and overall sensitivity. Among tested mixtures, the majority showed non-interactive (additive) toxicity. However, examples of both potentiation and antagonism were found. The occurred mainly in cases where there was a clear mechanistic basis for the observed effect, such as detoxification system inhibition of substrate competition. These finding suggest that, at least for initial assessment, current mixture models provide relevant indications of likely joint effects if uncertainty is included within the risk assessment.

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Interspecific sensitivity of wild bee species and the relevance of the honey bee as a surrogate organism for pollinators

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University of Landau, Institute for Environmental Sciences / Institute for Environmental Sciences
Honey bees are included in the European pesticide risk assessment as a surrogate species for all insect pollinators. In their bee guidance document EFSA proposed an additional assessment factor of 10 to account for interspecific differences in bee sensitivity. Furthermore, they recommended to include the buff-tailed bumblebee *Bombus terrestris* and the red mason bee *Osmia bicornis* into risk assessment. Since data on wild bee sensitivity is still scarce, there is a need for further investigation to evaluate if the assessment factor and the test species that EFSA proposed are adequate. We performed multiple dose-response contact toxicity lab tests with wild bees using dimethoate as a toxic standard. Afterwards, we generated a species sensitivity distribution (SSD), derived a hazardeous dose (HD5) and its lower 95% confidence limit (lower limit HD5). Furthermore, we determined the acute toxicity of multiple insecticides towards the proposed test species *O. bicornis* and compared it to honey bee data. HD5 was calculated to be 0.08 µg a.i./bee and the lower limit HD5 was 0.02 µg a.i./bee. The lower limit HD5 is equal to the mean 48h LD50 for *A. mellifera* calculated from literature data divided by a safety factor of 10. *B. terrestris* and *O. bicornis* were the least sensitive of all tested species. For 6 out of 9 additionally tested insecticides *O. bicornis* was more sensitive than *A. mellifera*. For dimethoate no further information is gained by conducting acute laboratory tests with the two wild bee species *B. terrestris* and *O. bicornis*. However for a range of insecticides *O. bicornis* seems to be more sensitive than the honey bee up to a factor of nearly 100. This generates questions of a suitable safety factor for acute risk assessment. Therefore, there is reasonable doubt that the honey bee is an adequate surrogate organism in pollinator risk assessment lower tier testing.

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Summary of an ICPPR Non-Apis Workshop - Subgroup Higher Tier (Bumble bees and Solitary bees) with recommendations for a semi-field experimental design
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The publication of the proposed EFSA risk assessment guidance document of plant protection products for pollinators highlighted that there are no study designs for non-Apis pollinators available. Since no official guidelines exist for semi-field testing at present, a protocol was proposed and a ringtest was conducted in 2016 to develop a general test set-up. The ringtest design was based on the EFSA guidance document, OEPP/EPPO Guideline No. 170 and results of discussions regarding testing solitary bees during the meetings of the ICPPR non-Apis workgroup in 2015 and 2016. Ringtests were conducted with two different test organisms, one representative of a social bumble bee species (*Bombus terrestris* L; Hymenoptera, Apidae) and one representative of a solitary bee species (*Osmia bicornis* L; Hymenoptera, Megachilidae). Both are polylectic and can forage on a diverse spectrum of flowering crops. Also, they are common species in Europe, commercially available and are widely used for pollination services. Several laboratories participated in the higher-tier ring test. 7 semi-field tests were conducted with *B. terrestris* and 8 semi-field tests were done with *O. bicornis*. Two treatment groups were always included in the ringtest: an untreated control and dimethoate as a toxic reference item (optional other i.e. brood affecting substances (fenoxycarb)). In the solitary bee study design adult bees were exposed in the tunnels during their reproductive period. The offspring was exposed to the treated pollen and nectar source during development. The hatching success of their progeny will be assessed in the following year. In the bumble bee study design only the early part of the colony development took place during the exposure phase in the tunnels. At the end of flowering, the bumble bee colonies were transferred to a monitoring site until they produced queens and drones ("switch-point"). Based on the results of the ringtest, main open questions will be answered, which are for bumble bees: how many replicates are needed to see possible effects? how can minimal variation of endpoints be achieved what are realistic variations in queen number and size/weight? how can the "switch-point" be defined reliably? how can the assessment of hatched queens be handled? for solitary bees: how can cocoon incubation and hatching of bees be synchronised with the onset of flowering? how fit are solitary bees out of season? which substance can be used as reference item for brood studies?

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Field and semi-field studies to evaluate the effects of foraging on thiamethoxam treated winter oilseed rape on the reproductive capacity of the red mason bee, Osmia bicornis
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To date, the honey bee *Apis mellifera* has been the primary test species used in environmental risk assessment and has been the main focus of work investigating the causes of pollinator declines, with fewer studies on bumblebees and solitary bees. It has been suggested that extrapolation of a risk assessment with honeybees to other bee species may not be appropriate as the level of exposure and response to pesticides may differ between species due to differences in sensitivity and ecology. Solitary bees have been included as test organisms with an associated risk assessment in the EFSA Guidance Document (EFSA, 2013), however this regulatory document is yet to be implemented. One key issue is the lack of validated test methods evaluating the potential risks of pesticides to solitary bees, both in the laboratory and field. These trials were conducted used a tailor-made design based on relevant publications and consultations with *Osmia* breeders and experts. The aim was to assess the chronic effects of thiamethoxam seed treated winter sown oil seed rape on the reproductive success of the solitary bee *Osmia bicornis.* The test organism was exposed under both semi-field and field conditions, with the semi-field included to ensure exposure of *Osmia* through foraging on a crop in a realistic worst-case exposure scenario. Field trials were conducted at three locations in Germany in 2014 and 2015. Each location included one control field (sown with untreated winter oilseed rape) and one treated field (sown with winter oilseed rape treated with 21µg thiamethoxam/seed, 2.5µg metalaxyl-M/seed and 0.6µg fludioxonil/seed) in both years. Adult red mason bees, *Osmia bicornis,* were exposed under field conditions for the duration of flowering each year. Additionally in 2015, tunnels were erected and *Osmia bicornis* were exposed under semi-field conditions. Parameters such as female nest occupation, cell production, foraging activity, and cocoon/offspring production were assessed. In addition samples of plants, flower, pollen and nectar and *Osmia* pollen mass were collected during the exposure phase for residue analysis of thiamethoxam and its metabolites CGA322704. The practicality of conducting studies with Osmia in field and semi-field scenarios was evaluated. Under the conditions of this experiment there was no evidence of any differences in the parameters assessed on the Osmia released in the control and treated field plots in either the field or semi-field studies.

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Example of an exposure field study handling three different pollinator species and several matrices of residue analysis
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The here presented study was set up in 2015 to determine residues and ecotoxicologically relevant concentrations (ERCs) of a plant protection product, classified as insecticide, in rapeseed (*Brassica napus*) inflorescences and their respective pollinator food matrices (i.e. pollen and nectar) followed by single application after daily bee flight activity. Application was conducted under field conditions and in terms of good agricultural practice on five different trials in Northern-western Switzerland at various times and locations. The maximum mean concentration of residues over time was determined in different matrices (e.g. nectar and pollen) collected by honey bee colonies (*Apis mellifera* L. (Hymenoptera: Apidae)), bumble bee colonies (*Bombus terrestris* (Hymenoptera: Apidae)) and solitary bee nesting cavities (*Osmia bicornis* (Hymenoptera: Megachilidae)). The exposure phase per field trial lasted for approximately 10-12 days on flowering *Brassica napus*. Over this period different matrices such as nectar, pollen and honey samples, depending on availability of the individual species, were taken for residue analysis. Further on sampling was conducted in a setup that the way of exposure / possible pesticide entry from field to hive could be demonstrated. The chosen species reflect an important part of the pollinator community in field and are therefore chosen for this field residue trial. The presented results and mode of action may be a significant addition and useful approach for creating further input and detailed data needed for the risk assessment on pollinators and their actual, realistic exposure to plant protection products based on the recent EFSA guidance document on the risk assessment of plant protection products for pollinator species (revised version July 2014).

Advancements in life cycle impact assessment and footprint method development (I)

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Linking environmental water requirements to freshwater biodiversity damage at multiple scales
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Freshwater ecosystems rely on the quantity, quality and timing of water flows to maintain their functionality, productivity and natural resilience. An ever-increasing freshwater demand results in the global degradation and loss of freshwater biodiversity. As a starting point to tackle this issue, the assessment of environmental water requirements (EWR) of ecosystems is needed. In this sense, several approaches have been applied at the local scale in many countries. Global models have been proposed and recently adopted in LCA to enhance the

environmental relevance of water stress indicators (e.g. AWaRe). Their strength lies in a relatively easy global applicability whereas they are not able to model ecological responses to water consumption. For this reason, mechanistic models to assess damage on ecosystem quality are still needed. The aim of this study is to reconsider the concept of environmental water requirements in light of these demands, by the application of habitat based effect factors and showing the potentiality for a large scale extension of ecosystem quality indicators based on EWR. Habitat simulation methods associate hydraulic variables with physical habitat suitability indicators to model target species responses to physical habitat change. Starting from local patterns of fish habitats, the potential aggregation of midpoint habitat indicators at the watershed level has been investigated. The analysis of midpoint habitat indicators at watershed level shows substantial uniformity in ecological responses for fish species with similar habitat preferences. For this reason, local habitat indicators can be aggregated at a larger scale. Species oriented midpoint and endpoint indicators (e.g. PAF and PDF) can be derived from species distribution data and considering the presence or absence of connected suitable habitats for those species affected by physical habitat alteration. Habitat mapping is a viable way to develop spatialized midpoint and endpoint indicators for water use impacts on ecosystem quality, deriving from mechanistic models based on EWR. At present this approach can be operational at the water/sub-watershed scale and PAF or PDF indicators can be evaluated in order to provide results comparable to those from other existing models assessing freshwater biodiversity loss. However, applying habitat based models at the large scale is necessary to provide LCA practitioners with global tools and ecosystem quality indicators.

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Assessing the impacts of hydroelectricity production on aquatic ecosystems in LCA: A multi-scale approach

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Many large dams are being constructed in developing economies, or are in operation to provide hydroelectricity, which is a renewable and low carbon emissions energy source. However, the regulation and fragmentation of river caused by dams may impact ecosystem functions and biodiversity. At the moment, Life Cycle Assessment (LCA) do not consider the impacts of hydroelectricity production on aquatic ecosystems. In this project, we are developing scientifically sound characterization factors quantifying the impacts of hydroelectricity production on aquatic ecosystems. More specifically, we evaluated the impacts on fish communities across several spatial scales, from a global (worldwide patterns) to a local scale (detailed boreal ecosystem dataset) . We examined change in species richness (i.e., number of species, which relates to the so-called *Potentially disappearing fraction of species* (“PDF”) in LCA), and also examined changes in species composition, relative abundance and absolute abundance (which relates to the *Potentially affected fraction of species* (“PAF”) in LCA). Preliminary results suggest that no significant impacts on fish richness could be related to hydroelectricity production in boreal and temperate zones (i.e., “PDF” = 0). However, a decreasing trend observed in the tropics suggest that some regionalisation is necessary in LCA to develop characterization factors. At the local scale, using a dataset from boreal reservoirs, we observed a generalized change in species composition in sites located upstream of the dam following impoundment. A closer examination of abundance pattern at the population scale suggest that some species significantly benefited from impoundment but some were detrimentally affected (“PAF” > “PDF”). Overall, we suggest that using species richness and “PDF” as a standalone metric of anthropogenic impacts on fish communities in LCA might be insufficient since species richness and “PDF” measures are insensitive to change in fish species composition. We suggest complementing traditional “PDF” metrics with “PAF” for a more comprehensive characterization. Regionalization might be needed when developing characterization factors in LCA because the impact of hydroelectricity production on fish species differ in relation to the latitude, some characteristics of the reservoirs and their management, and the complexity of species trophic interactions in reservoirs.

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Marginal or non-marginal contribution to water scarcity footprint: different approaches

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Water Footprinting methods in LCA have gained a lot of attention in the recent years and their applications have increased significantly. A consensus building process took place lead by the WULCA group of the Life Cycle Initiative and recommended a method for global assessment of water scarcity footprint. The uptake of such a method by the community sometimes lead to applications that are outside the scope of the designed indicator, or any previously used scarcity index in LCA. These indicators were meant to address the more traditionnal LCA-based assessment of a marginal contribution to water consumption, i.e. one that does not change the background. When large-scale assessments, such as the water scarcity footprint of a country or of a large development project are foreseen, different factors based on different hypothesis reflected in the modeling are needed When considering potential impacts caused by water consumption, a curve similar to a dose-response curve in toxicity lies behind the modeling. The assessment of a marginal water consumption assumes no change in the slope at the point of current local water consumption level, i.e. a negligible change in background water consumption. If a large, already occurring, water consumption is to be assessed, such as a nation-wide water consumption, this assumption does not hold and the already occurring impacts should be redistributed to all occurring water consumption, by considering the area under the curve, hence taking the integral. A third approach consists of assuming that each cubic meter consumed in a region potentially generates the same additional impacts, whether it is the first or last, and hence disregards any impact curve. With this approach, the area under a straight line is considered and the total potential impacts distributed equally to each consumed cubic meter. These approaches are presented and the hypothesis behind are discussed. Although consensus was reached on one method to assess water scarcity footprint in LCA, it is important that the method be used within the context for which it was designed, and when less traditional LCA used are assessed, adapted factors are required. Different proposals are being developed for different purposes and this paper contributed to shed light on some of them and the philosphies behind.

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A new indicator for product water availability footprint

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Water availability footprint can be influenced by both consumptive water use (related to water removal from a water body/catchment) and degradative water use (related to emissions affecting water quantity). In the current practice of water availability footprint assessment, the critical dilution approach to assessing degradative water use is not satisfactory and rules of processing background data from existing life-cycle-assessment (LCA) databases are needed. This study aims at presenting a new indicator for product water availability footprint. The water availability footprint indicator (WAF) is defined as the sum of results for consumptive water use (V_{con}) and degradative water use (V_{deg}). V_{con} equals the volumetric change between water extraction and wastewater effluent. V_{deg} for emissions i is defined by the emission load and the reference emission concentration in a water quality standard. V_{con} and V_{deg} for background processes are calculated based on specific rules/assumptions for processing data from existing databases. The WAF is then multiplied by the water stress index (WSI) to get a weighted indicator WSAF, followed by aggregating every WSAF into a life-cycle score. The case study is a cradle-to-gate analysis of cotton jeans in China. Wastewater treatment processes of denim weaving/denim finishing/jeans washing contributes to freshwater availability even when water availability footprints associated with their material and energy inputs are considered. Thus, a wastewater treatment process that purifies water quality can increase freshwater availability and should not be ignored, nor should its WAF score be set to be 0. For every unit process, the indicator score varies with the assessed emission. The scores of WAF and WSAF for biochemical oxygen demand are larger than that for chemical oxygen demand. It is not reasonable to mix indicator scores of different emissions, for example, by adding up many unit-process scores that are calculated as maximum grey water footprints of different emissions. We presented the new single stand-alone indicator WSAF and demonstrates its feasibility in the case study of cotton jeans in China. The primary benefit added by WASF to product water availability footprint assessment lies in its ability to distinguish water purifying processes (e.g. wastewater treatment) from water degradative processes as well as its operability with existing LCA database support.

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Poster spotlight: TU208, TU209, TU210, TU211

Looking across organizational boundaries: exchanging ideas on mechanistic modelling between SETAC and the International Water Association (IWA) (I)

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Overview of major topics from SETAC & IWA

i. nopens, Ghent University; K. Villez, Eawag - Swiss federal Institute of Aquatic Science and Technology; E. Zimmer, IBACON GmbH; T. Gouin, Unilever / Safety and Environmental Assurance Centre

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Developments in Integrated Urban Drainage
L. Vezzaro, DTU (Technical University of Denmark); W. Rauch, University of Innsbruck

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Developments in Wastewater Treatment
P. Steen Mikkelsen, Technical University of Denmark / Department of Environmental Engineering; i. nopens, Ghent University

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Developments in Drinking Water Treatment
B. Martijn, PWN Technologies
This work illustrates the technological aspects to the application of MP UV/H2O2 treatment as a non-selective barrier for organic micropollutants in advanced drinking water production from eutrophic and polluted surface water. Furthermore, the mechanism and the preliminary risk of MP UV nitrate photolysis induced byproduct formation was studied, although the identity of the formed reaction products could not be established. The TEF concept, a widely accepted approach for risk assessment of mixtures of compounds with a similar mode of action, was used in this study to perform a MOE based preliminary risk assessment of the mixture of unknown compounds with genotoxic mode of action, causing the positive Ames test response in MP UV treated water. The preliminary risk assessment based on the MOE approach, using the developed 4-NQO based TEF, exceeded the level of no concern. However, MP UV/H2O2 treatment as part of an integrated multibarrier water treatment scheme provides a robust barrier for organic micropollutants and allows mitigation of formed byproducts.

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State-of-the-art tools for exposure and chemical risk assessment
D. Van de Meent, Independent Consultant; J. Struijs, Radboud University Nijmegen; D. De Zwart, DdZ Ecotox / Centre for Sustainability Environment and Health
The EU chemicals' regulation REACH demands that chemicals be registered before they can be marketed. REACH registration dossiers should include a Chemical Safety Assessment, demonstrating that (and how) chemicals can be used safely; the REACH guidance provide guidelines for doing so. The REACH Guidance published by the EU Chemicals Agency (ECHA) lists the modeling instruments that have been reviewed by international expert groups and found useful for this purpose. Prominent REACH instruments are multimedia mass balance models that describe and predict the exposure concentrations that are expected in the environment, given the normal uses and market volumes listed in the registration dossiers. Among these instruments are the sewage treatment simulation model SimpleTreat and the air/water/soil exposure assessment model SimpleBox. In their decision-support system KnowSEC, the German Federal Environment Agency (UBA) has recently combined these two models into what is provisionally named the 'simple^{2}boxtreat', to model so-called 'Exposure Potentials' of chemicals. Exposure potentials express the expected proportionality between market volumes and concentrations in the environment, assuming 'normal use', and given properties of the chemicals (among which their proposed uses) and characteristics of the receiving environment (assuming the uses as proposed).\n This presentation illustrates the use of the REACH models SimpleTreat and SimpleBox, as combined in UBA's 'simple^{2}boxtreat' and discusses some of the model changes that have recently been proposed to ECHA by the Dutch National Institute of Public Health and the Environment (RIVM), following recommendations by the European Centre For Ecotoxicology and toxicology of Chemicals (ECETOC).

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Mechanistic effect models for ecological risk assessment: where next?
V. Ducrot, Bayer CropScience AG / Environmental Safety Ecotoxicology; P. Thorbek, Syngenta / Environmetal Safety; T. Preuss, Bayer Ag / Environmental Safety
Mechanistic effect modelling has been discussed for decades in (eco)toxicological research as a tool to predict effects of chemicals at the individual, population and community levels. Recently, the European Commission and EFSA have recognized the relevance of these tools for use in the context of pesticide risk assessment, as shown by the inclusion in recent guidance documents and scientific opinions risk assessments for birds, mammals, aquatic animals/plants, soil organisms, bees and non-target arthropods. Models are suggested to improve the understanding of the mechanisms driving effects and recovery patterns and to extrapolate to different exposure scenarios, seasons, geographic zone and species, as well as to link endpoints from standard regulatory studies to specific protection goals. In order to facilitate the use and evaluation of such models by notifiers and risk assessors, EFSA has published a scientific opinion on good modelling practice, which discusses the steps needed to achieve good models (e.g. calibration, verification, validation) and some principles for how to use these models in regulatory risk assessments as well as methods to address the model uncertainty. Notifiers already use these models in support of pesticide risk assessments. First examples of use suggest that a modular approach where various building blocks or "modules" can be assembled together in a flexible manner to form the regulatory model best allows to ensure reliable model predictions for the risk assessment at hand (in contrast to a "one model fits all" approach). Authorities are getting more familiar with reviewing modules, models and conclusions from model-based assessments. However, the level of acceptance of these assessments still varies widely, depending on the experience of the authority in using the models and its overall confidence in the modelling approach. Factors that could favour a wider acceptance and use of models for regulatory purposes are (i) the development of an EU agreed modelling toolbox with standardised building blocks and rules to assemble them in order to create a fit for purpose regulatory model (with e.g. adequate space and time scales) (ii) agreement on ecological scenarios (iii) agreement on model validation strategies and (iv) an open discussion of model uncertainty and model-based risk assessment uncertainty. Achieving this level of harmonisation and standardisation will require reinforcing the stakeholder engagement and dialogue in the future.

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Integrating treatment facility and river network information to model spatially-explicit environmental concentrations of down-the-drain substances: iSTREEM
C.M. Holmes, R. Vamshi, Waterborne Environmental, Inc.; P. DeLeo, D. Ferrer, American Cleaning Institute; S.D. Dyer, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization
iSTREEM® is a web-based model which estimates spatially-explicit environmental concentrations of down-the-drain chemicals in effluent and receiving waters across the USA. Concentrations are estimated at the discharge points of over 10,000 municipal wastewater treatment plants (WWTPs) and downstream receiving waters covering more than 350,000 km of rivers. The model incorporates WWTP information on population served, treatment type, and facility flow which are linked to a commonly used hydrology framework providing flow and hydrologic connectivity between facilities and downstream sites. As part of the hydrologic routing, a first-order decay is implemented to simulate environmental processes that remove chemical from the water column. The model allows for regional use rates to better simulate potential geographic variability in emissions, as well as differing removal rates to account for different facility treatment types. Given the assumption of temporally constant emission, the model is able to efficiently execute as a single, annual model run. The publicly available web-based model (www.iStreem.org) exemplifies open access to modeling resources, with no software installation required, and computation resources for model runs performed by the iSTREEM server. Users are able to save and retrieve runs, interact with results in a map format, or download source data and model results for more in-depth analysis by the user, including linking to desktop mapping software. The model, sponsored by the American Cleaning Institute (ACI, www.cleaninginstitute.org), is a valuable tool for both promoting product and ingredient stewardship and potential regulatory compliance for chemical suppliers and manufacturers of formulated products. The framework and modular nature of the model allow it to be applied to different geographies beyond the current USA-wide dataset.

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Cost-Effective and Integrated Optimization of the Urban Wastewater System Eindhoven
L. Benedetti, Waterways; T. Flameling, Waterschap De Dommel; J. De Klein, Wageningen University / Aquatic Ecology and Water Quality Management; J. Langeveld, Partners4UrbanWater; i. nopens, Ghent University; S. Weijers, Ghent University / BIOMATH
The Dommel is a small sensitive river flowing through the city of Eindhoven, receiving discharges from a 750,000 PE WWTP and 200 CSOs. In summer time, the WWTP effluent is up to 50% of the river base flow, which does not yet meet the requirements of the European Union Water Framework Directive (WFD). Waterschap De Dommel, the responsible authority for this compliance, has launched a comprehensive project to find the most cost-effective set of measures (Real Time Control (RTC), additional treatment capacity, sewer and CSO adaptation) for meeting the WFD requirements. An integrated model was developed to find cost-effective and sustainable integrated urban water system solutions to increase the water quality of the river, and it includes: for the sewers, a tanks-in-series (TIS) hydraulic model is a simplified version of the detailed sewer models of all systems in the ten involved municipalities with simplification at spatial level, lumping catchments and modelling only significant pipes and

overflows; for the WWTP, the model is the same as the detailed one, while the water quality model will not need simplification as it is not the computationally demanding part of the IUWS model; for the river, also a TIS model is made for hydraulics, and the water quality model is the same as the detailed one; the spatial discretisation depends on the significant inputs and on the river hydraulics. The above described models have been implemented independently, calibrated and validated using data from simulations of the detailed models. Subsequently, they have been integrated (after developing specific interfaces to translate state variables between models) into a single executable model. This approach allows overcoming: the communication problems between different software platforms, reducing the possible scenarios to be run that require true integration, especially regarding integrated RTC simulation speed problem of the detailed models, allowing to reduce the time needed to run each (long term) scenario by several orders of magnitude. This model is a key element in the 4M-approach (Monitoring, Measuring, Modelling, Measures) that we apply to determine the most cost-effective set of measures. It enables finding an optimum that combines best use of the available infra-structure through RTC with additional innovative measures. The solution found is significantly more cost-effective than conventional solutions to increase the water quality of the river.

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Alternative approaches to animal testing for (eco)toxicity, and the regulatory application of the 3Rs in chemical risk assessments (II)

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Benchmarking study to support regulatory acceptance of the application of in vitro-in vivo extrapolation to predict bioaccumulation potential of chemicals
H. Laue, Givaudan Schweiz AG / Fragrances S & T; H. Gfeller, L. Hostettler, Givaudan Schweiz AG; K. Jenner, Givaudan; G. Sanders, Givaudan International SA / Regulatory Affairs and Product Safety; A. Natsch, Givaudan Schweiz AG / Fragrances S &T
Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment of chemicals. The bioconcentration factor (BCF) is usually determined in fish (OECD TG 305), but alternative approaches are encouraged by the European Chemicals Agency to avoid new vertebrate testing. *In vitro* systems measuring biotransformation rates of chemicals to refine BCF model estimates have been established as alternative methods to refine predictive models which are based on hydrophobicity (i.e. log K_{ow}). Whereas the reproducibility/reliability of these *in vitro* assays was recently proven in a multi-laboratory ring trial to support the development of two OECD test guidelines (OECD project 3.13), more data are needed to show the relevance of the *in vitro* assays to support the regulatory acceptance. The goal of this study was to compare BCF predictions based on measured *in vitro* intrinsic clearance rates in trout liver S9 fractions to BCF estimations using classical models for 20 fragrance ingredients (log K_{ow} 3.9 to 6.5) with *in vivo* BCF data. Additional chemicals from other use categories, which are known to be bioaccumulative, were tested as controls. Substrate depletion was analysed by GC-MS. The biotransformation rates then served as input for an *in vitro-in vivo* extrapolation (IVIVE) model to extrapolate a whole-body biotransformation rate constant which is used to predict BCFs. A broad range of *in vitro* intrinsic clearance rates was found for the 20 fragrance molecules ranging from 0.2 to 25.3 ml/h/mg protein. The majority of the fragrance molecules were moderately to rapidly transformed. No significant turnover was observed with either Musk xylene or the control chemicals with a reported high bioaccumulation potential. Predicted BCFs for fragrance molecules with relatively high log K_{ow} values (>4.9) were closer in agreement with *in vivo* BCFs when the correction factor f_U used to estimate fraction of chemicals unbound was set to 1. These predicted BCFs obtained with the IVIVE model closely reflected the *in vivo* values. Most importantly, these estimations did in general not lead to underprediction of the BCFs, but avoided the strong overpredictions due to classical log K_{ow}-based models. *In vitro* S9 metabolism data in combination with the refined *in vitro-in vivo* extrapolation model are a valuable tool to assess bioaccumulation potential as part of a weight of evidence approach and can be included as an additional level of screening in the evaluation of B.

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Screening for low aquatic bioaccumulation: prediction potential for replacing BCF tests based on physicochemical properties
M. Nendza, Analytisches Laboratorium; R. Kühne, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; A. Lombardo, IRCCS - Istituto di Ricerche Farmacologiche Mario Negri; S. Strempel, ETH Zurich; G. Schuurmann, Helmholtz centre for environmental research - UFZ / Institute for Organic Chemistry
Aquatic bioconcentration factors (BCFs) are critical in PBT and risk assessment of chemicals. High costs and use of more than 100 fish per standard BCF study (OECD 305) call for alternative methods to replace as much in vivo testing as possible. The BCF waiving scheme is a screening tool based on physicochemical properties (hydrophobicity, air/water partitioning, biodegradability, hydrolysis, ionisation) to predict substances with low aquatic bioaccumulation (nonB, BCF < 2000). The nonB compounds cannot classify as PBT, and, hence, their bioconcentration testing may be waived because of low concern with regard to the B criterion. The BCF waiving scheme was developed with a dataset of reliable BCFs for 998 compounds and externally validated with another 181 substances. It performs with 100% sensitivity (no false negatives) and 60% efficacy (waiving potential). The chemical applicability domain (AD) of the BCF waiving scheme is given by the structures of the training set, with some compound classes explicitly excluded like organometalls, poly- and perfluorinated compounds, aromatic triphenylphosphates. The prediction confidence increases with (1.) the number of physicochemical criteria triggered, (2.) the distance of the physicochemical properties from the thresholds, and (3.) the similarity with the chemical structures with either low or (very) high bioaccumulation (BCF ≥2000). The classification statistics for the compounds of the training set inside the AD the BCF waiving scheme give an overall accuracy of 68%. Sensitivity values for B and vB of 100% (no false negatives) demonstrate the protective nature of the BCF waiving scheme. The performance of the BCF waiving scheme is even better for compounds of the external validation set inside the AD. The overall accuracy of the BCF waiving scheme is 82% and no false negatives are detected (100% sensitivity). The classification efficacy shows that at least 60% of the truly negative nonB compounds inside the AD may be identified already at early levels of their assessment and, hence, are candidates for waiving of experimental BCF studies due to low concern with regard to the B criterion. The BCF waiving scheme allows to reduce the bioacumulation tests with fish by at least 60%. External validation confirms good prediction confidence if the domain of applicability is regarded. Chemicals not covered by the models require further assessments.

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Facilitating Mechanistically Based Grouping to Predict Acute Aquatic Toxicity Using Information from Molecular Initiating Events
M. Sapounidou, Liverpool John Moores University / Pharmacy and Biomolecular Sciences; M. Cronin, J. Madden, Liverpool John Moores University; S. Gutsell, Unilever / SEAC; G. Hodges, Unilever / Safety and Environmental Assurance Centre SEAC
Mechanistic approaches may assist in hazard assessment and the categorisation of compounds to allow for read-across to reliably fill data gaps. Existing classification schemes for the assignment of mechanism of action aquatic toxic action (e.g. Verhaar, Russom, Barron) are employed widely. However, they have limited coverage since they are based on restricted chemical domains and, predominantly, on fish toxicity studies. To extend the utility of the currently available schemes, the aim of this study was to develop a classification scheme for industrial organic compounds that incorporates molecular initiating events, as defined by known and putative Adverse Outcome Pathways (AOPs), related to acute toxicity to a diverse set of aquatic species. Molecular Initiating Events (MIEs) were defined and characterised for established mechanisms of action (e.g. electrophilic reactivity, neurotoxicity, AChE inhibition, etc), where possible with regard to AOPs, for a range of aquatic species. Robust, high quality, acute toxicity data for eight aquatic species (e.g. *Oncorhynchus mykiss*, *Daphnia magna, Pseudokirchneriella subcapitata*) were analysed. More than 80 MIEs were subsequently clustered into one of three groups: a) non-specific toxicity (narcosis); b) non-specific reactivity; c) specific toxicity. Chemical domains were assigned for each MIE, subsequently forming the chemical domain of each group. The current approach provides a flexible schematic map of MIEs to facilitate hazard identification for acute aquatic toxicity; will enable interspecies extrapolation; and can be expanded to chronic toxicity within the overall framework of reliable AOPs.

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The Effluent Toxicity Assessment Toolbox - International Perspective on Tools and Concepts and Opportunities for Animal Alternatives
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Effluent testing has been utilized in many countries to assess potential ecological

impacts and assist in determining necessary treatment options for environmental protection. However, it was only relatively recently that toxicity-based effluent assessments and subsequent discharge controls became globally important, when it was recognized that physical and chemical measurements alone did not protect the environment. Various strategies using different toxicity tests and whole effluent assessment techniques, plus supporting analytical tools, have been developed. Concurrently, interest in reducing animal use has risen. A workshop was held in March 2016 to evaluate concepts and tools for effluent assessments and update the toolbox of for effluent testing methods. The objectives were to identify opportunities to use a suite of strategies, identify opportunities to reduce reliance on animal tests, and determine barriers to implementation of new methodologies. Effluent approaches and methods must address both short and long-term adverse effects, persistence, and bioaccumulation, and should also allow for chemical monitoring of specific parameters, bioassessment, and consideration of exposure and the receiving environment. Use of novel and alternative test methods should be integrated when appropriate; however, there is a need to derive consistent criteria for developing these new approaches. To meet animal protection goals, a toolbox approach that integrates use of modelling, invertebrates, and other alternative monitoring methods are essential. These novel approaches will also allow for the opportunity to examine specific, non-traditional endpoints, such as EDCs, genotoxicity, teratogenicity and other specific MOAs. This talk will highlight the workshop, including a review the state of the science, description of the suite of strategies being used for reducing the impact of effluents, discussion on ways to integrate alternative approaches, and identification of opportunities to reduce the reliance on animal testing. Finally, we will give an overview of how various countries approach effluent testing and discuss how these approaches and data generated can integrate into existing risk assessment methodologies. *The views, conclusions and recommendations expressed are those of the authors and do not necessarily represent the policies or positions of the European Commission, Environment and Climate Change Canada or the US Environmental Protection Agency.*

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Poster spotlight: TU001, TU002, TU003

Marine and freshwater ecotoxicology (I)

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Endocrine biomarkers in bivalves - lessons learned so far
J. Morthorst, H. Holbech, K. Pedersen, University of Southern Denmark / Department of Biology; P. Bjerregaard, University of Southern Denmark / Biology
Vertebrate endocrinology is highly conserved across classes and the effects of endocrine disrupting chemicals (EDCs) have been intensively studied in vertebrates such as fish. Less is known about the effects of EDCs on invertebrates. To understand better the effects of EDCs on molluscs the development of valid biomarkers is requested but unfortunately, detailed knowledge about regulation of bivalve reproductive endocrinology is scarce. It is absolutely essential for the reliability of a biomarker to have properly optimised and validated detection assays and to have an in-depth understanding of both fundamental biology and the underlying mechanisms. Therefore painter's mussels (*Unio tumidus*) and blue mussels (*Mytilus edulis*) were exposed to 17β-estradiol (E2) and 4-t-octylphenol (4-t-OP) in the laboratory, to investigate if yolk protein synthesis could be induced by compounds with known estrogenic effects in vertebrates, and *U. tumidus* were collected over the course af a year in a Danish uncontaminated lake to investigage annual variations in hemolymph yolk protein levels. Species specific Enzyme Linked Immunosorbant Assays (ELISA) were developed for yolk protein in *M. edulis* and *U. tumidus,* and the gonads were investigated by histologyto establish annual reproductive cycles and to identify intersex. When determined by ELISA male yolk protein levels of *U. tumidus* are fairly constant throughout the year whereas female yolk protein levels increase significantly in February and March. In the period before the increase male and female levels are not significantly different. Yolk protein levels of male and female gonads of *M. edulis* are also similar in the resting phase. Female yolk protein levels in fish are several orders of magnitude higher than male levels, and that is one of the conditions for using yolk protein induction as estrogenic biomarker in fish. Based on our findings, that yolk protein is not induced by classic vertebrate estrogens/xenoestrogens and the lack of sexual dimorphism in bivalve yolk protein levels, we have learned that yolk protein of bivalves has a completely different role compared to fish, and even validated biomarker assays for fish cannot be directly transferred for use in other species. With the currently available knowledge, it is strongly recommended not to use induction of yolk protein as estrogenic biomarker in bivalves.

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Application of label-free shotgun proteomics to identify and quantify vitellogenin in marine mussels
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Vitellogenin (vtg), the egg-yolk precursor in female oviparous animals, is used as a biomarker of estrogenic endocrine disruption due to its induction by xenoestrogenic pollutants, and specially to its anomalous appearance in males. One commonly used method for the estimation of vtg levels is the measure of alkali labile phosphates (ALP). This indirect method is based on the analysis of phosphate released from the phosphorylated proteins present in the sample under the assumption that vtg is the most abundant phosphorylated protein there. This seems to work well with fish serum, however in mollusc this method has been questioned due to the major presence of other phosphorylated proteins in different tissues and haemolymph. Therefore, it is necessary to develop a more accurate technique to measure directly vtg in marine mussels. In this study, we have applied shotgun proteomics using high resolution LC-MS/MS to identify and quantify vtg in mussel gonads. The aim of this work is to standardize a technique for the measurement of vtg in the gonads of the marine mussel *Mytilus galloprovincialis*, and to check whether vtg synthesis is induced after exposing individuals to a synthetic estrogen: 17α-etynilestradiol (EE2). Mussels from an uncontaminated area were collected at two gametogenesis stages (early gametogenesis and fully mature) to quantify vtg levels in males and females. Additionally, mussels in early gametogenesis stage were exposed to the synthetic hormone 17α-etynilestradiol (EE2) for 4 days to assess whether vtg synthesis was induced by estrogenic compounds. Shotgun proteomics identified and detected vtg only in female gonads, while it was detected in both genders in ALP analysis, with even higher levels in mature males than in females. These results indicate that the ALP method is not a suitable proxy for measuring vtg levels in marine mussels. Results related to the vtg induction after EE2 exposure are, at the moment, inconclusive. That is, vtg induction in EE2 exposed mussels was observed but the increment compared to control group is not statistically significant. Additional samples are being analyzed in order to increase the statistical power of our analysis, as well as to assess the influence in the observed results of gonad maturation stage of mussels as a potential confounding factor.

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The effects of copper-cadmium mixtures on the development of zebrafish embryos
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For some time metals have been substances of global concern, due to their toxicity and their wide spread occurrence in the environment from both natural as well as anthropogenic sources. And while substances almost never occur on an individual basis in the environment there is still a large gap in the understanding of mixture toxicity and it is therefore a subject of great interest. So far there has been limited success in trying to understand and predict the interactions of mixtures. In this study we aim to bring more understanding to metal mixture toxicity by investigating the effects of Cu and Cd on the development and survival of the zebrafish embryo. The toxicity was initially evaluated on an individual basis. Afterwards the embryos were also exposed to a full factorial design of 18 binary mixtures. The effects were assessed for up to 120 hours post fertilization (hpf) with an extensive final scoring at 120 hpf including the following endpoints: mortality, heart beat count (24hpf), hatching, distinction of body parts, spine curvature, oedema, blood accumulation, swim bladder inflation. In addition to being scored on the basis of morphological endpoints the hatched zebrafish were also video tracked for 50 minutes in order to assess the effects of the metals and metal mixtures on swimming behaviour. Full dose-response curves were obtained for both metals on the basis of mortality as well as swim bladder inflation, one of the most sensitive sub-lethal endpoints. The results showed that zebrafish embryos had very steep mortality dose-response curves for both metals with Cu being the more toxic of the two metals (LC_{50} Cu 135.6 µg/L, LC_{50} Cd 3262 µg/L). On the basis of swim bladder inflation the observed responses were less steep and unlike the curves observed for mortality Cd was found to cause effects at lower concentrations than Cu (EC_{50} Cu 34.46 µg/L, EC_{50} Cd 10.12 µg/L). Using the individual dose-response data mixture predictions were made using the Independent Action (IA) model and compared to the observed mixture mortality. The comparisons of the Cu-Cd mixture exposure observations and predictions on the basis of several of the evaluated endpoints, including mortality and swim bladder inflation showed clearly synergistic interactions.

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Effects of environmental pollutant mixtures on immune function and disease susceptibility in wildlife
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Persistent organic pollutants (POPs) are known immunotoxicants in laboratory animals and a growing weight of evidence suggests similar effects occur in wildlife

exposed to these chemicals. Outbreaks of distemper and influenza A virus in marine mammals causing death of thousands of animals over the past decades has risen concern about the role that environmental pollutants may have in reducing the immune system's ability to combat infection. To better elucidate the effects of environmental pollutants on immune and disease outcomes in wildlife, we performed two sets of *in vitro* exposure experiments using realistic pollutant mixtures. Firstly, we performed dose-response experiments for mitogen stimulated lymphocyte proliferation, natural killer (NK) cell activity and phagocytosis using live cells collected from polar bears (*Ursus maritimus*), bottlenose dolphins (*Tursiops truncates*), harp seals (*Pagophilus groenlandicus*), and hooded seals (*Cystophora cristata*). Secondly, we studied the effect of pollutant cocktails on disease outcomes using influenza A infections in Madin-Darby canine kidney cells (MDCK). Chemical cocktails extracted directly from marine mammal adipose tissues contained a suite of POPs, including polychlorinated biphenyls (PCBs), organochlorinated pesticides, and several legacy and current-use brominated flame retardants. Lymphocyte proliferation was dose-dependently suppressed in all species while NK activity was enhanced at the highest exposures; phagocytosis was not modulated at tested concentrations. Surprisingly, pollutant cocktails dose-dependently decreased influenza infectivity in MDCK cells. This observation was linked to complex interactions between viral infectivity, cellular innate immunity and chemical stress, assessed via targeted gene expression and functional assays. Together, these results significantly advance our understanding of the impacts of global pollution on wildlife and reduced overall health due to pollutant exposure-modulated immunosuppression.

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UK MSFD assessment: spatial and temporal analysis of biliary 1-hydroxypyrene, ethoxyresorufin-O-deethylase (EROD) and acetylcholinesterase (AChE) activity in flatfish

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Pollution of the marine environment by chemicals from anthropogenic sources can cause long term effects on the ecosystem. Detecting harmful chemicals in water, sediment or biota as part of a monitoring scheme will tell us what is there but not how it is affecting the species present in the ecosystem. Biological effects techniques can give us a clearer picture of what classes of contaminants the species sampled have been exposed to and from this we can assess how healthy our marine environment is. Descriptor 8 of the Marine Strategy Framework Directive (MSFD) requires monitoring of biological effects of contaminants in the marine environment. As part of the Clean Seas Environmental Monitoring Programme (CSEMP) data has been collected for EROD activity, biliary PAH metabolites and AChE activity in flatfish (Dab *Limanda limanda*, plaice *Pleuronectes platessa* or flounder *Platichthys flesus)*. This data collected by Cefas and MSS has been examined for trends to see if sites around the UK are at or below baseline levels, improving or degrading over the past 10 years. EROD and AChE activity and PAH metabolite concentrations were assessed against the corresponding OSPAR Background Assessment Criteria (BAC). Environmental Assessment Criteria (EACs) were used to indicate whether unacceptable effects may be occurring. Responses between BAC and EAC indicate exposure to contaminants, but significant environmental impact is not occurring. Where there were no breeches of the EAC, or results were below BAC, this indicates that these sites are not heavily contaminated with chemicals that cause these biological responses in flatfish. For those sites with an upwards trend further monitoring may be required to ensure they do not exceed the EAC in the future. Sites which are considered 'clean' may be monitored less regularly but should still be examined in case of future pollution events, and to maintain a baseline in a changing climate. The results of this analysis will be used to inform regulators on the state of the UK marine environment.

Engineered nanomaterial effects on soil and terrestrial communities

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Exposure dynamics of metallic nanomaterials (Ag, ZnO, Cu and Pb) affect soil microbial functional diversity

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Metallic engineered nanomaterials (ENMs) have been widely applied in cosmetics, medical devices, clothing and food industry. They are likely to end up in soil and sediments through wastewater effluents and deposition. The aggregation and accumulation of the non-degradable ENMs will inevitably impose increasing pressures on soil environment. ENMs are known to affect bacterial processes and metabolic activities. Most of the tests rely on initial exposure concentrations and did not take the dynamic dissolution of ENMs into consideration. Moreover, the toxicity to functional responses of soil microbial communities is caused by the particulate forms or the ion forms of ENMs remains poorly understood. We investigated the dynamic changes of exposure of ENMs (Ag, ZnO, Cu and Pb) in relation to their impact on the functional diversity of soil microbial communities. We applied the response addition model and concentration addition model to explicitly evaluate the joint toxicity of the particulate forms and the ion forms of ENMs. The dynamic exposure of ENMs were considered using time weighted average concentration (TWA) approach. In a series of experiments, we found that expressing the dynamic exposure of ENMs based on TWA yields more realistic and strict EC_{50} values compared to using initial concentrations. The toxicity of spherical ZnO NPs, spherical CuNPs and SMPs, and Pb-based perovskites were significantly higher based on TWA concentrations compared to the toxicity based on initial concentrations. In addition, the particulate forms for Ag, ZnO and Cu ENMs were the main factors affecting soil microbial functional diversity at EC_{50}, while the Pb ions released from perovskites were predominance in the effect of perovskites on microbial communities. Our results thus hint that the time-variable exposures of ENMs disrupt metabolic processes of soil microbial communities and in turn their associated ecosystem processes, and both the dynamic exposure of ENMs and relative toxic contributions of particles and ions are essential to understand in pursuing a naturally realistic assessment of environmental risks of ENMs.

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Unraveling the effects of soil components on the toxicity of AgNP to F. candida (Collembola) - an acute toxicity test in aqueous medium

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The use and application of silver nanoparticles (AgNP) has increased in recent years and with it the release to the environment. Soils are considered major sinks of AgNP in the environment but many questions related to the fate of AgNP in soils still remain. Various studies have shown that soil characteristics such as pH, texture and ionic composition influence the toxicity of AgNP to soil biota. We chose to focus on the standardized components of the OECD soil (peat, sand, kaolin clay) in our experiments as a first step to deeper understand the fate, bioavailability and toxicity of AgNP in soil. Collembola are important contributors in breakup of organic material in soils. The species *Folsomia candida* is used in an OECD guideline to evaluate the effects of chemicals on the reproduction of Collembola. In this test the exposure of Collembola to contaminants is via soil particles and pore water. We developed a simplified soil pore water (SPW) system in which the contact of the Collembola to AgNP is only via the aqueous route. SPW was simulated by preparing test vessels with 0.3 ml soil components and 2 ml of spiked water solutions. Four animals per vessel were placed on the surface of the aqueous phase for two weeks. At the end of this period the immobilization in each treatment was determined. In the aqueous phase, ionic and particulate silver was distinguished. We hypothesize that (a) the fractions of particulate and ionic silver will vary with respect to the soil components, (b) the interactions of soil and silver components are concentration-dependent, and (c) the forms of silver in the simulated pore water affect the toxicity. Part of the experiments are currently still running but first results indicate that peat decreased the portion of immobilized Collembola in presence of AgNP. In sand and kaolin mortality increased compared to the respective water controls without AgNP. Chemical analyses of the simulated pore water show that the bioavailable fraction of silver is higher in sand than in other soil components, and is mainly composed of nano-Ag rather than ionic Ag. This indicates that the toxicity of AgNP is due to nano-effects. We will analyze further toxicity and analytical data to develop a mechanistic model of toxicity of AgNP to Collembola via SPW exposure in OECD soil. The parameters of this model can be used as a first basis for developing model predictions of AgNP toxicity in differently composed natural soils.

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Long-term ecotoxicological and genetic effects of engineered nanomaterial exposure in the springtail Folsomia candida

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Serious concern has been raised about the potential entry and impact of engineered nanomaterials (NMs) on aquatic and terrestrial biota since the beginning of the nanomaterials industry, not more than a decade ago. To date, studies on engineered NMs have mainly been performed on aquatic biota. Only few studies have reported the effects of NMs on terrestrial soil invertebrates, with only two studies investigating ENM toxicity in *Folsomia candida*. Previous toxicity studies with *Folsomia candida* have mainly used the single-generation test with survival and reproduction as endpoints. This may underestimate the potential effect of the toxicant, as multigenerational exposure could lead to a delayed reproductive failure, bio-accumulation passed on to the offspring or an accumulation of DNA damage. Therefore, the aim of this study is to determine the effects of WCCo and CuO nanomaterials on survival, reproduction, and gene expression for four consecutive generations, plus an additional two generations in clean soil to study recovery. *F. candida* did not show any effects on reproduction when exposed to CuO NMs at concentrations as high as 6400 mg/kg dry soil. However, at gene expression level we observed induction of Laminin A protein essential in tissue development and maintenance. The WCCo NMs did affect reproduction although only at very high exposure levels. Interestingly, the animals became more sensitive at the 3rd generation, with the highest concentrations (1600, 3200 and 6400 mg/kg) showing a significant reduction in reproduction compared to the control. The WCCo NM EC50 was twofold lower when compared to the 1st generation. Upon incubation for two generations in clean soil, after 4 generations of exposure, complete recovery was seen. For animals that were exposed to WCCo NMs, gene expression of metallothionein was induced at the highest levels of exposure. Apparently, metal detoxification is triggered through metallothionein. This study shows the importance of multigeneration testing at different levels of biological organization to explain environmental impact of engineered NMs on soil invertebrates.

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Copper toxicity in Collembola: a comparison between nano and non-nano agrochemicals
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Nowadays one of the biggest challenges is to answer to the world´s growing demand for food and energy and agriculture sector is increasingly being pressured to be more efficient and sustainable. Over the past decade nanotechnology has increasingly been used in agriculture specially in the form of nanopesticides. These products raise valid concerns about their fate and behaviour in soils and aquatic systems due to their extensively use and applicability. The objective of this work was to compare the toxicity of a copper nanopesticide (Kocide 3000) with other non-nano copper pesticides and their correspondent a.i. and related it to the compounds behaviour and porewater bioavailability using the model organism *Folsomia candida*. Reproduction tests were performed following the standard guideline for springtails and were ran in two soils, Lufa 2.1 and 2.2 with two times for spiking equilibrium (0 and 48 hours). Results showed differences in toxicity between the copper a.i. alone and the commercial formulation. The nanopesticide Kocide 3000 shows a similar toxicity trend when compared to the correspondent non-nanopesticide, nevertheless $Cu(OH)_2$ is found to be more toxic than the other copper forms. When exposures in the different soils were compared, we can see an increase of toxicity in Lufa 2.1, probably due to a lower fitness performance of collembola related to the higher availability of Cu^{2+} in soil by increasing solubility. The spiking times revealed a tendency to higher values of EC50s in the 0h equilibrium time and porewater analysis presented different copper content depending on the copper form tested and also dependent on the day of porewater collection. This study will help to improve hazard assessment of metal nanomaterials but also to better understand nanopesticides behaviour in soil and their toxicity towards terrestrial organisms exposed via porewater. Keywords: nanopesticides, soil, porewater, springtails

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Do we worry too much? Nanoparticle and other stressors' effects to a soil-grown legume at realistic exposure levels
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Engineered nanomaterials (ENMs) are relatively novel contaminants of emerging concern entering the soil environment *via* a broad variety of waste streams, and are currently a hot topic for novel nano-pesticides and -fertilizers in agriculture. Therefore, ENMs are increasingly scrutinized for their (eco)toxicity to plants. It is yet unknown how ENM exposure compares to other stresses such as drought, exposure to conventional chemicals, or insufficient micronutrient status. Such information is needed to put the effects and potential hazard of nanoparticles in context with other environmental stressors, and to better understand the mode of toxic action of ENMs. In this study, we used gold nanoparticles (nano-Au) as a non-dissolving model nanoparticle to compare ENMs and different environmental stressors (micronutrient silicon status, drought, and citrate as a stabilizing agent for the nano-Au and conventional relatively non-toxic chemical) in a soil-grown agricultural legume at realistic exposure concentrations. The effects of the absence and presence of the micronutrient silicon, of the drought, of the nano-Au, the combination of Si + nano-Au, and citrate was quantified and characterized by an interdisciplinary approach including X-ray fluorescence microscopy (μ-XRF), computed micro-tomography, gene expression analysis, and evaluation of macroscopic plant parameters such as drought resistance and biomass. As a whole, the findings show that an agriculturally relevant legume exposed to a ENM (nano-Au), even at subacute concentrations in the low $\mu g\ kg^{-1}$ range, exhibited anatomical changes similar to the adaptation of plants to conventional stresses. The comparison with other conventional stressors and a micronutrient shows that the ENMs under investigation (which did not leach toxic metals) did not cause more effects than a common micronutrient, drought stress, or low concentrations of citrate considered as safe. Apparently, strong defense reactions and physiological adaptations of higher plants could effectively cope with the ENMs in the present study. Only if several stressors are combined, more adverse outcomes may be possible. [1] Schwab F, Zhai G, Kern M, Turner A, Schnoor JL, Wiesner, MR. 2016. Barriers, Pathways and Processes for Uptake, Translocation, and Accumulation of Nanomaterials in Plants—Critical Review. Nanotoxicology 10, (3), 257-278.

Experimental approaches and field studies to investigate ecosystem integrity under multiple stress

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Pesticide effects in a standardized and repeatable aquatic tri-trophic Nanocosm
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Agricultural pesticides are often used to enhance crop production; however, besides acting against targeted organisms also non-target organisms might be affected. The effects can be both direct and indirect when interactions with other system components are altered. To date, chemical impacts are routinely assessed in rapid single-species tests that fulfil regulatory needs for standardization, replication and repeatability. Effects on ecological interactions can be studied in multi-species tests, however, these impacts are often neglected. Reasons for this are the complexity, the difficulty of standardisation and the resource demand of many multi-species tests. Also, they are often low in statistical power due to a limited number of replicates and growing variability between them. As a result, risk assessment is often restricted to the effect assessment at the individual level even though growing evidence exists that effects on individual organisms are not directly proportional to impacts at the population and the community level. The use of ecologically more relevant testing procedures has thus become a priority to risk assessors and regulators. We developed an aquatic, tri-trophic Nanocosm (algae, *Ceriodaphnia, Hydra*) as an intermediate link between simple single-species tests and complex multi-species systems, focusing on the ability to detect small stressor-induced alterations in ecological interactions. The starting conditions and sampling techniques were optimised to fulfil the needs for standardization, repeatability and replication. We exposed the Nanocosm community to different concentrations (e.g. RAC, EC10) of a herbicide (Linuron) and determined the impacts on system components and their interactions. Here we show how the population dynamics and the interactions between species were altered by the herbicide and illustrate how the results compare to the effects measured in single-species tests.

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Pesticide toxicity is enhanced by pre-exposure to wastewater
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Leaf-shredding macroinvertebrates contribute substantially to the breakdown of leaf litter, an essential ecosystem-level process in many low-order streams. At the same time these ecosystems often act as recipients of treated municipal wastewater (WW) containing complex mixtures of micropollutants. While the detrimental effects of chronic exposures to WW on shredders are well studied, little is known about how these exposures affect shredders' tolerance to other (chemical) stressors. For instance, many low-order streams also receive pesticide loads from adjacent agricultural areas and pesticide toxicity may be assumed to be higher when shredders were pre-exposed to WW. To experimentally test this hypothesis, we pre-exposed the amphipod shredder *Gammarus fossarum* for up to 6 weeks to stream water, WW, or a 50:50 mixture of both, while monitoring their survival and leaf consumption. After 2, 4, and 6 weeks of pre-exposure, animals' physiological fitness was determined and 7-days lasting dose-response experiments with the model pesticide thiacloprid were conducted using leaf feeding activity as endpoint. Both pure WW and the 50:50 mixture significantly reduced gammarids' leaf consumption during the first 4 weeks of pre-exposure, while also survival was significantly reduced at all (50:50) or the last two sampling dates (WW) by up to 60%. While gammarids' physiological fitness was only negatively affected in animals exposed to WW after 6 weeks of exposure as indicated by a significantly lower dry weight compared to the control, WW pre-exposure clearly enhanced pesticide toxicity in a dose-dependent manner: animals from the WW treatment were always most sensitive towards thiacloprid followed by animals from the 50:50 mixture. Our data thus indicate that pre-exposure to WW can enhance shredders' sensitivity towards a model pesticide by up to 2.5 times, which seems particularly worrying given that exposure to WW in the field can be year-long and may thus even further impair these animals' capabilities to withstand additional stress such as exposure to pesticides. Over the long term such exposures may, however, either

result in physiological or genetic adaptation or trigger changes in the species composition of shredder communities. Therefore, it seems mandatory to understand these species assemblages' long-term adaptational mechanisms to WW exposure and their consequences in a "multiple stress" environment to ensure that functional integrity is safeguarded.

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Effects of fungicide exposure and feeding pressure on aquatic fungi across different biogeographical regions

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Fungicides are the most frequently used pesticide group in the EU. Non-target aquatic fungi, which play a key role in organic matter decomposition (OMD), are repeatedly exposed to fungicides and simultaneously subjected to feeding pressure by shredders. Previous studies combining fungicide exposure and macroinvertebrate shredders to study effects on OMD mainly analysed short-term effects, while effect propagation over several fungal generations has not been assessed yet. We investigated the combined and individual effects of fungicides and feeding pressure on the ecosystem function OMD over three fungal colonisation cycles in Germany, Sweden, Denmark and Australia. After colonising leaf bags of the first set in unpolluted streams, we conducted a six-week-lasting experiment with three consecutive leaf sets (i.e., runs), which were colonised by the related earlier leaf set. Four treatments were tested with six to seven replicates: one control and three levels of a fungicide mixture (Pyrimethanil, Prothioconazole, Metalaxyl; concentrations: ΣTU-3, ΣTU-2, ΣTU-1), with a two-day peak, followed by a 12-day base exposure of field-relevant concentrations. The experiment was run with and without local shredders (gammarids in Germany and Denmark, caddisfly larvae in Sweden, no local shredders were available in Australia). At the end of each run, the following endpoints were analysed: OMD, fungal community structure and fungal biomass. OMD strongly differed among the countries in which the experiment was conducted (four factorial ANOVA, p

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Effects of fungicides and nutrients on heterotrophic microbial communities under global change projections - the importance of exposure history

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Ecosystem functions provided by microbes in pristine low-order streams (e.g., leaf litter breakdown) may be globally threatened by increasing fungicide and nutrient stress due to an intensified agricultural activity. Effects may, however, be less severe in streams already affected by agriculture. To test this, we studied the effects of a fungicide mixture (0, 6, 60, 600 μg/L) and nutrients (0.2, 2, 10, 18 mg NO_3-N/L) on the structural and functional plasticity of leaf-associated microbial communities with differing exposure histories (pristine vs. agriculture) in a 2×4×4-factorial design (n=6) for 21 d. Leaf breakdown rates (LBR) as well as fungal species richness, community composition, and biomass were chosen as dependent variables. Under fungicide exposure, LBR differed significantly between the communities (P=0.031): While LBR for the pristine community reduced by up to 30% upon fungicide exposure compared to controls, the agricultural stream community increased LBR by up to 85%. This pattern became more pronounced with increasing nutrient levels, indicating a supporting effect of nutrients on the agricultural stream community that partially compensated for fungicide stress (up to 150% higher LBR in controls at high compared to low nutrient levels). For the pristine community, however, this positive effect (up to 40% higher LBR) was suppressed, likely by strong fungicide-induced stress. Distinct structural effects may trigger this pattern. First, pre-exposure towards fungicides and elevated nutrient levels in the field may have induced a pollution-induced community tolerance in the agricultural stream community explaining the maintained function under stress. Second, the observed shift in fungal community composition associated with a significant reduction in species richness (P< 0.001) led to the dominance of few species in the agricultural stream community for which higher LBR compared to other fungi were reported. Third, the supposedly tolerant fungal species of the agricultural stream community produced an up to 40% higher biomass than their pristine counterparts under fungicide stress. Given the correlation between biomass and LBR, it is likely that this observation contributed to the maintained function. Since pristine ecosystems are globally under pressure by an agricultural expansion, results point at a broad scale loss of biodiversity and ecosystem functions at the base of food webs that ultimately jeopardizes the integrity of stream ecosystems.

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CASCADING AGRICULTURAL CONSTRAINTS ON PLANT LITTER FUELED TROPHIC LINKAGES

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Organic matter (OM) serves as a food source sustaining diverse food webs and is governed by a complex interplay between its chemical composition, physical abrasion, microbial decomposition and consumption by invertebrates. Leaves from terrestrial plants often constitute one of the major OM-inputs in aquatic ecosystems, and therefore can dictate the quantity and quality of OM available for decomposition and consumption. Aquatic systems are thus prone to changes driven by land use activities at adjacent fields. Moreover, agricultural practices involve the intensive use of pesticides and fertilizers. These chemicals are poorly soluble and therefore quickly become associated with sediment organic particles, and hence a complex mixture of organic particles, inorganic pollutants, and OM-biocide complexes is detected. These OM-complexes are subsequently subject to physical and biological processes (e.g. photolysis; microbial decomposition and macrofaunal digestion) that further complicate the chemical attributes of these OM-complexes, making it difficult to predict and experimentally identify realistic effects of anthropogenic activities on ecosystem functioning. In a series of experiments, we demonstrate that anthropogenic pressures such as agricultural practices can constrain microorganisms and invertebrates living in adjacent drainage ditches. These constraints are the result of complex trophic interactions and ultimately result in an impaired functioning of the ecosystem. These studies exemplify the interconnectedness of bacteria and invertebrates and highlight the importance of sub-lethal effects and trophic complexity of anthropogenic pressures on ecosystem functioning.

Bioremediation and phytoremediation of contaminated environments

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DEVELOPING ECO-INNOVATIVE CHEMICAL PROCESSES TO VALORISE PHYTOREMEDIATION-BORNE BIOMASSES : THE PHYTOCHEM INITIATIVE

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Industrial and urban activities impact our environment, especially in terms of soil pollution. During the last two decades, gentle soil remediation techniques have emerged using various plant species and the combination of microbial biotechnologies. Several phytotechnologies can be applied to produce useable biomass, alleviate pollutant linkages and restore services of polluted soils: (1) phytostabilisation, which uses perennials able to sorb and immobilize trace elements (TE) in excess in the root zone, avoiding their transfer toward groundwater and aerial parts and preventing their bioaccumulation in the food chain as well as dispersion by natural agents (2) phytoextraction, based on root-to-shoot transfer and storage of TE in harvestable plant parts. The PHYTOCHEM project aims at promoting chemically-based valorisation processes of the plant biomasses collected from polluted sites managed by eco-innovative phytotechnologies. As a first step towards the development of chemically-based valorisation processes of plant biomasses collected from polluted sites, we have successfully implemented 16 and 18 woody species at two phytomanagement sites in 2014 (Figure 1). Based on ICP-AES analysis, TE concentrations in aboveground tissues highly varied, illustrating the metal tolerance processes developed by plants, either by exclusion or accumulation. We further applied chemical processes to contaminated biomasses. For instance, optimization of an organosolv pre-treatment allowed for removing ca. 50% of Fe and Mn from contaminated biomasses, while Ecocatalysis was also successfully applied to i.e. Zn hyperaccumulating species (A. halleri).

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From bioavailability science to bioremediation of PAHs: where are the limits for risk reduction?

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Polycyclic aromatic hydrocarbons (PAHs) are the best representatives of chemicals for which specific limitations in bioremediation exist due to low bioavailability. The residual concentrations of the PAHs after bioremediation are crucial because they may limit the use of the area after treatment, or land use might not even be possible if the residual concentrations do not meet the legal requirements. For bioavailability to be incorporated into soil bioremediation, three questions must be addressed: (1) how is "bioavailability" defined? (2) how should it be measured? and (3) is it possible to increase bioavailability but not environmental risk of the pollutants? Over the last 30 years, numerous publications have discussed the concepts and definitions of bioavailability of organic chemicals (*Environ. Sci. Technol.* 49:10255-10264, 2015). The main schools of thought consider bioavailability (focusing on the aqueous or dissolved contaminant), bioaccessibility (incorporating the rapidly desorbing contaminant in the exposure), and chemical activity (determining the potential of the dissolved contaminant for biological effects). These concepts are the basis for different methodologies (desorption extraction, passive sampling and biological tests) and mechanistic studies that consider the different processes that are involved (contaminant soil/sediment interactions, transport and passage across cell membrane, and biological responses such as toxic effects or biodegradation). Our group has proposed different ways to operate at different levels on these processes, in the context of biodegradation of PAHs, for a better bioremediation performance in risk reduction. The approach is relevant because in some circumstances bioremediation may even increase risk of PAHs. The prospected risk-minimizing strategies include (bio)surfactant-enhanced slow-desorption (*Environ. Sci. Technol.* 45:3019-3026, 2011; *Environ. Sci. Technol.* 48:10869-10877, 2014), the targeted fertilization of free-oil phases or NAPLs (*Environ. Sci. Technol.* 45:1074-1081, 2011), modulated deposition and motility of microbial degraders in porous media (*Environ. Sci. Technol.* 46:6790-6797, 2012), promotion of bioavailability with plants and root exudates (*Soil Biol. Biochem.* 57:830-840, 2013; *Environ. Sci. Technol.* 49:4498–4505, 2015), and facilitated microbial (bacterial/oomycete) interactions for the formation of biofilms and colonization of pollutant interfaces (*Sci. Total Environ.* 511: 767-776, 2015).

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Plant-assisted bioremediation as an effectiveness strategy to remediate a historically PCB and heavy metal-contaminated area in Southern Italy

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Plant-based clean up technologies are gaining popularity as sustainable solutions to contaminated soil remediation. In particular, plant-assisted bioremediation or phyto-assisted bioremediaton exploits the synergistic action between plant root system and natural microorganisms (bacteria and fungi) to remove, convert or contain toxic substances in soil and water. It relies on the use of a selected appropriate plant for stimulating in the rhizosphere (e.g. through root exudates production, oxygen transport) the biodegradation activity of natural soil microorganisms. In this context, a poplar-assisted bioremediation strategy has been applying for three years to a multi-contaminated (PCBs and heavy metals) area in Southern Italy using a specific poplar clone (*Monviso*). It was chosen thanks to its capabilities previous tested for promoting hexachlorocyclohexane degradation. At selected times (0, 420, 900 days) PCB and heavy metal (HMs: V, Cr, Sn, Pb) concentrations were assessed on soil samples at different depths and distance from tree trunks inside some contaminated plots. Similarly, microbial analyses were performed on soil samples to assess total microbial abundance, cell viability, dehydrogenase activity and the phylogenetic composition of the autochthonous microbial community. Three years after the poplar planting a significant decrease in PCB and HMs concentrations was observed. Currently, the values of all PCBs detected are under the Italian legislation limits in the plots investigated. The microbiological analysis show an overall improvement in soil quality in terms of an increase in microbial abundance, cell viability and organic carbon content in the rhizosphere soil samples. Moreover, the phylogenetic analysis of the microbial community showed a higher percentage of *Bacteria* in the rhizosphere than in the bulk soil. In particular, a significant increase in *Actinobacteria* and *Alpha-Gamma-Proteobacteria,* which include several speciesable to degrade PCBs, was observed. Overall results show that the poplar-assisted bioremediation strategy was able to promote both the persistent organic contaminant degradation and the phytostabilization of the inorganic ones.

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Role of rhizoremediation in a complex PCB soil contamination gradient

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In the last two decades there has been a growing interest in bioremediation technologies which use plants and microorganisms to degrade organic chemicals such as Polychlorinated Biphenyls (PCBs) in contaminated sites. These techniques represent a good alternative to traditional remediation technologies, being cheaper, not disruptive and more suitable for large contaminated areas. Different studies have been conducted to investigate the potential of plant-microbe interactions in the remediation of organic chemical contaminated soils with respect to natural attenuation, providing useful data such as chemical degradation rates (K_D) or half lives (HL). Such a type of data can be used to predict soil concentration temporal trend, as well as the time needed to achieve legal limit when using plants and their associated rhizosphere microbe to remediate contaminated sites. In the present work, rhizoremediation experiment derived K_D or HL for PCBs will be used as input parameters in an existing dynamic air-plant-litter-soil model (SoilPlusVeg) to estimate PCB concentration temporal trend in the soil of a National Relevance Site (SIN) for remediation located in Northern Italy (SIN-Brescia Caffaro). Analyses of sample collected in this area have shown the presence of a high spatial variability of PCB concentrations with values varying up to 3-4 orders of magnitude. Therefore it could be interesting to understand if rhizoremediation might be a suitable technique for the entire site. A number of long term simulations were run with SoilPlusVeg model for some PCBs and results were used to 1) compare the influence of natural attenuation vs. plant/microbe interactions on soil concentrations, 2) evaluate the effectiveness of rhizoremediation when a complex contamination gradient is present. Simulations results showed that although the importance of natural attenuation vs plant/microbe interactions depends on PCB physical chemical properties, when considering rhizoremediation experiment derived degradation rates the time requested to achieve legal limits decrease. However, rhizoremediation must be accurately implemented (in terms of species to be selected, their density, etc) to account for effective remediation, especially when complex patterns of contaminant are present.

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Recycling of waste water treatment sludge as a substitute soil amendment for mine waste reclamation

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The last two fluorspar mines in France, closed in 2006, were opencast sites (Montroc and Le Moulinal) that cover a combined 165 hectares, 30km from Albi in southwest France. Natural colonization of the backfilled open pit and waste rock piles has been minimal due to a combination of low pH (4.5) and high residual concentrations of fluoride, manganese, iron and associated trace metals (copper, zinc and lead). In order to minimize dispersion of metals in leachate, a novel solution has been adopted, namely to recycle the alkaline (lime) residues derived from an on-site water (leachate) treatment plant as a neutralizing surface amendment, and as a partial substitute for topsoil. A pilot lysimeter experiment was undertaken on the Mont-Roc and Le Moulinal substrates to test the water treatment residue (0 % - control; 1%; 20% and 50 % v/v) : (1) as a means of pH correction, (2) to immobilize the indigenous metals and fluoride, and (3) prevent them from reporting to groundwater in leachate. The four treatments were also subject to two bioassays: glasshouse trials using *Lotus corniculatus* and *Festuca arundinacea* for productivity tests, and the Rhizotest root mat technique (ISO 16198) to assess phytoavailability of trace metals in the alkaline residue-treated mine waste. The pilot experiments evidenced a very positive effect of the water treatment residues on pH correction, and decreased metal and fluoride concentrations in the leachate. Moreover, plant bioassays reflected markedly improved conditions for plant growth, and much reduced bioavailability of metals and fluoride. These encouraging results provided the basis for field trials at the two sites (Mont-Roc and Le Moulinal) with the objective of proving the fitness of this innovative recycling technology for developing a sustainable vegetation cover.

Environmental Fate, Effects, and Risk Assessment of Veterinary Medicines

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Updates to the proposed Canadian regulatory framework for the environmental assessment of new active ingredients in human and veterinary drugs

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A Canadian regulatory framework has been developed specifically for active ingredients in human and veterinary drugs regulated under the *Food and Drugs Act* (F&DA) to assess potential risks to the environment and indirectly to human health resulting from environmental exposure. This regulatory framework has been designed to harmonize with the drug approval process stipulated by the F&DA and its regulations. Health Canada developed this framework in collaboration with stakeholders including Environment and Climate Change Canada, industry and environmental non-government organizations. The framework was endorsed in principle by stakeholders in 2011. Since then, the proposed regulatory framework has been modified to increase alignment with environmental assessment approaches in other jurisdictions and to incorporate recent technical developments related to the environmental assessment of active ingredients in human and veterinary drugs. Highlights include a proposal to adopt VICH guidelines 6 and 38 for the environmental assessment of active ingredients in veterinary drugs, and updates to screening level exposure assessments such as new Canada specific defaults for predicting environmental concentrations of veterinary drugs in soils. The proposed changes will be consulted on in early 2017. The purpose of this presentation is to present the revised regulatory framework being proposed.

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Estimating soil emissions and toxicity impacts from the application of livestock manure: application to heavy metals at national scale
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As a source of nutrients and organic matter, livestock manure is commonly applied on agricultural land for its fertilizing potential. It however contains traces of veterinary medicines and feed additives such as antibiotics and heavy metals that might cause toxic impacts on the environment. Environmental risk assessment (ERA) and life cycle assessment (LCA) are two tools that enable to evaluate such environmental risks and impacts and can support regulatory purposes. Both first require an estimation of the releases made to the environment in the respective phases of exposure assessment (in ERA) and life cycle inventory analysis (in LCA). Here, we propose a general methodology for estimating toxic emissions to soil at national, regional and global scale based on the quantities of manure applied and their average pollutant content. A global inventory was built for emissions of arsenic, cadmium, chromium, copper, mercury, nickel, lead and zinc for 215 countries between 2000 and 2014. Time series revealed that developing countries apply an increasing amount of manure and thus proportionally release more heavy metals to their agricultural land, while emissions are stable or decreasing in developed countries. Using life cycle impact assessment, it was found that mercury, zinc and copper are the substances contributing the most to impacts on human health and on freshwater ecosystems. When normalizing the toxic impact by the area of cultivated agricultural land, island countries obtained high scores followed by European countries and South East Asian countries. The study highlighted the sensitivity of methods used to measure the heavy metal content of manure and the need to establish standardized sampling and analytical methods to allow for better country differentiation and data comparison. Building such inventory is expected to help assess the pollution of agricultural soil due to the application of manure and may support national or sectoral policy-making such as regulating the concentration of heavy metals in manure for land application.

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Challenges in exposure modelling of fish veterinary medicines
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The currently available guidelines for the environmental impact assessment (EIA) for veterinary medicinal products (VMP) (VICH GL 38, VICH GL 06 and the 2008 revised guideline)[2] [3] [4] describe a general assessment framework for the assessment of environmental risk to aquaculture VMP, although there is no technical guidance as to the derivation of refined Predicted Environmental Concentrations (PECs). The 2008 guidelines[4] specifically recommend the use of the models designed by the Scottish Environmental Protection Agency (SEPA) for the regulation of the fish farming industry[1]. In the context of EU regulations, the acute risk associated with use of the aquaculture veterinary medicinal product is evaluated at Tier A, while the chronic risk is evaluated at Tier B. In order for the SEPA models to fit into this framework, it is proposed to use the short-term model at Tier A and substitute 3h- and 6h- environmental quality standards (EQS) with acute PNEC values, and to use the long-term model at Tier B and substitute 72h-EQS with chronic PNEC values. Defining standard regional conditions is another challenge for Environmental Risk Assessments of aquaculture VMP. It is difficult to define generic site conditions, and site-specific assessments are more appropriate to the nature of the SEPA models. One option is to run the models many times over a range of expected environmental conditions to identify safe uses, although direction is currently lacking as to which combination of farm parameters and site environmental conditions can be considered a realistic worst case. \n References:\n [1] SEPA. 2008. Regulation and monitoring of marine cage fish farming in Scotland - a procedures manual. Annex G: Models for assessing the use of chemicals in bath treatments v2.2 [2] VICH. 2000. Guideline on Environmental Impact Assessment for Veterinary Medicinal Products - Phase I, CVMP/VICH/592/98-FINAL. [3] VICH. 2005. Guideline on Environmental Impact Assessment for Veterinary Medicinal Products - Phase II, CVMP/VICH/790/03-FINAL. [4] VICH. 2008. Revised guideline on Environmental Impact Assessment for Veterinary Medicinal Products

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A new approach for supporting the comparison of the benefits and environmental risks of veterinary medicinal products
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Veterinary medicinal products (VMPs) require, as part of the European Union (EU) authorization process, consideration of both risks and benefits. The use of VMPs has multiple risks (e.g. risks to the animal being treated, to the person administering the VMP) including risks to the environment which are analyzed in this presentation. Environmental risks are not directly comparable to therapeutic benefits; there is no standardized approach to compare both environmental risks and therapeutic benefits. We have developed methodologies for communicating and comparing therapeutic benefits and environmental risks for the benefit-risk assessment that supports the EU authorization process. Three approaches (i.e. classification, relative classification, and a visual scoring matrix classification) were developed to categorize risks and to compare them to benefits. We applied these 3 approaches to the risks of 3 case-study VMP compounds (an anthelmintic, an antibiotic, and a non-steroidal anti-inflammatory drug) using summaries of product characteristics, literature sources, and regulatory guidelines. Comparisons were highly limited by differences in data sets; however, testing highlighted the advantages and disadvantages of each of the approaches to informed decision-making. Improving these approaches requires comparable data sets (e.g., same endpoints, level of assessment, exposure modeling scenarios) and comprehensive information on benefits such that similar levels of information are available for both risks and benefits. However, use of any of these approaches, even without the above improvements, particularly if applied prior to the implementation of risk mitigation measures, will support the authorization (i.e., decision-making) process, providing greater certainty to regulators, industry, and stakeholders. This research is being built on by further research regarding the application of risk-benefit assessments on the farm scale.

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Substitution in the context of the European authorization of veterinary pharmaceuticals
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The substitution principle is a critical element of modern chemical regulation in Europe. If implemented properly, it introduces incentives to continuously decrease the use of particularly hazardous chemicals and processes. It is therefore applied during the authorization of plant protection products, biocides and for industrial chemicals that are flagged as substances of very high concern. In sharp contrast, the substitution principle is not considered at all during the authorization of veterinary medical products. We therefore present suggestions on how the current regulatory approaches can be extended, in order to ensure that the use of active substances of high environmental concern is reduced to the minimum necessary, and only as long as no less environmentally hazardous alternatives are at hand. Similar to the procedures in the aforementioned regulatory frameworks, we suggest to use the PBT properties of an active substances as a criterion for identifying candidates for substitution. Critical issues that will be discussed during the presentation are (i) possible amendments of the current regulatory framework, (ii) the identification of alternatives, (iii) hazard vs. risk-based approaches, (iv) the necessary comparative hazard and risk assessment and their data demands, and (iv) the resulting need to time-limit market approvals. Ivermectin, Diclofenac and Tylosin and their possible alternatives will be used to illustrate these points.

Interpreting Biological Effects of Metals and Their Mixtures in the Aquatic and Terrestrial Environment (II)

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Chronic metal mixture toxicity to freshwater organisms: does the metal concentration ratio matter?
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Metal mixture studies often investigate mixture toxicity at equitoxic combinations (i.e. same toxic unit for each metal), which may not be representative for environmental metal concentration ratio's. Therefore, interactive effects using equitoxic metal concentration ratios may not be simply extrapolated to environmentally realistic exposure situations. However, it is at the moment not clear if this also applies to aquatic organisms. The present study had two main research aims. First, we investigated if mixture effects are different between studies with *environmental* and *equitoxic* metal concentration ratio's. Second, we evaluated to what extent metal concentration ratios used in aquatic chronic metal mixture studies deviate from metal concentrations actually observed in the environment. First, we investigated the chronic toxicity of ternary Ni-Zn-Pb mixtures to *Ceriodaphnia dubia* in 6 different waters. For each water metal mixture toxicity was investigated using both an *equitoxic* ray (combining metals at equitoxic

concentration ratios) and *environmental* ray (combining metals at concentration ratio's measured in the Dommel, a tributary of the Meuse in the Netherlands). Mixture effects were evaluated relative to the concentration addition model. Second, we evaluated whether the metal concentrations used in chronic metal mixture studies are environmentally relevant, we conducted a review of the literature for chronic freshwater metal mixture studies. Metal concentration ratio's in chronic experiments were compared to a set of four European monitoring databases (FOREGS, VMM, Dommel and UK). Our results showed that the metal mixture effects in *equitoxic* rays were always antagonistic relative to the concentration addition model, while mostly non-interactive in *environmental* rays. This suggests that chronic metal mixture effects observed in *equitoxic* experimental designs can not be simply extrapolated to *environmentally* realisticconcentration ratio's. Dependent on the metal combination considered, metal concentration ratios in many chronic metal mixture experiments to date tend to deviate considerably from measured metal concentration ratios in various European freshwater scenario's. Our study highlights that metal concentration ratios need to be carefully chosen to increase the environment relevance of chronic metal mixture studies.

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Context Matters: What Is Known about the Additivity of Toxicity in Ni-Containing Metal Mixtures
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The additivity or non-additivity of the toxicity of metal mixtures to aquatic organisms is becoming of increasing concern as regulators begin to progress from regulatory frameworks mostly based on individual chemicals to frameworks based on the interactions of multiple chemicals. To help inform that process, we conducted a literature review of the toxicity of Ni-containing metal mixtures in laboratory aquatic-toxicity tests. We located 104 laboratory toxicity studies from 1946 through 2016, in which aquatic organisms were exposed to at least one Ni-containing metal mixture. Sixty different combinations of metals were tested, ranging from 1 to 13 other metals combined with Ni. A large majority (82%) of the combinations of publication and water type used some form of actual or reconstituted freshwater. Fish were tested most often (35%), followed by invertebrates (23%) and individual algal species or phytoplankton communities (15% combined), with every other major taxonomic group < 10%. Dissolved organic carbon (DOC) concentration, an important toxicity-modifying factor, was reported in only 10% of the publications; and only 8% of the publications reported temperature, pH, alkalinity, hardness, major anions and cations, and DOC. Measured metal concentrations (either total or dissolved) were reported in only 36% of the studies. The mixture toxicity was reported as less-than-additive, additive, and/or more-than-additive, depending on the metals in the mixture, their concentrations, the exposure-water chemistry, the species of aquatic organism tested, and the authors' definition of additivity. Consequently, the additivity of the toxicity of Ni-containing metal mixtures is context-dependent and difficult to generalize. Additivity could be an acceptable assumption at a screening level in a tiered assessment, but might lead to undesirable over-protection or under-protection at more-refined levels of assessment. A better approach in higher tiers would be to use bioavailability-based metal-mixture toxicity models to predict the direction and extent of metal interactions. However, to support development/refinement of such models, authors need to report complete water chemistry (e.g., temperature, pH, alkalinity, and concentrations of DOC and major geochemical anions and cations) and toxicity data, which is still not the case in some current metal-mixture toxicity publications.

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Are interactions between organisms relevant to assess toxicity of metal mixtures in wastes? A TME approach
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The use of organic wastes as soil amendments is an alternative to landfilling which improves soil properties. However, besides organic contaminants, metal mixtures are often found in wastes and, for that reason, limit values for metals in these matrices have been established in European countries. These limits, however, consider only single metals, not taking into consideration effects resulting from interactions between elements. On the other hand, standardized single species ecotoxicological tests are useful to understand the potential toxicity of metals in wastes since metal mixtures are assessed as a whole. These tests have also been used to validate predictive models (e.g. concentration addition model) for metal mixtures. However, the interactions between species within a community (that are not considered in single species tests) may influence the toxicity of metal mixtures (through cascade effects) and, therefore, should be considered. Terrestrial Model Ecosystems (TMEs) approaches provide a more holistic view of the impact of wastes on soil organism communities. The present study aims to evaluate the influence of organism interactions from terrestrial ecosystems in the toxicity of two metal contaminated wastes (a compost and a digestate). To reach this aim, a TME approach was followed and toxicity data on soil activity (through bait-lamina), mesofauna diversity and Enchytraeids abundance were obtained in several sampling times. These data were compared with literature toxic values obtained from single species ecotoxicological tests for the test wastes and for the different metals present in test wastes. The results obtained suggest that soil communities are not affected by the test wastes. However, when considering toxic values for metals obtained from single species ecotoxicological tests reported in the literature and metal contents of test wastes, toxic effects would be expectable for mesofauna, especially on enchytraeids and collembolans. When exposed to metal mixtures through organic matrices in a real scenario, results indicate that mesofauna seems to be much more resistant and/or resilient than expected.

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Understanding the effects of complex (and realistic) metal mixtures to soil invertebrates
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Metal contaminated sites are a global environmental concern, in particular due to mining activities, and result from the accumulation and persistence of toxic metals in soils. As a result metals have been relatively well studied both in terms of their toxicity and chemistry. However, most of this research has been performed with single metals which contrasts with contaminated sites where metals occur in mixtures. In soil ecotoxicology some research has been performed observing the effects of metal mixtures on soil invertebrates but, most of this research, has focused on binary and tertiary mixtures and on only one standard test species. This approach does not adequately translate the complexity of metal contaminated sites nor addresses different biological routes of exposure. In this study we assess the effects of complex, five element metal mixtures, on the reproduction of three standard test organisms (*Folsomia candida*, *Enchytraeus crypticus* and *Oppia nitens*), using a fixed ratio ray design. Fixed ray designs use a constant ratio of different metals across increasing doses and the fixed mixture rays are selected to maximize risk interpretation. Results processed so far find that there are differences in organism sensitivity to metal mixtures and also species specific deviations from the concentration addition model. In particular for one of the tested soils, antagonism was observed in *E. crypticus*, whilst both *Oppia nitens* and *Folsomia candida* responses followed concentration addition. These preliminary results demonstrate the need to test complex metal mixtures including different species as a way to understand the role of biology in terms of intrinsic sensitivity and routes of exposure affecting metal uptake. Further analysis is underway for different soils, analyzing dose and ratio dependent deviations from concentration addition and results are currently being validated by measuring total and extractable metal concentrations as well as organism body burdens.

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Use of a biotic ligand model (BLM) to predict toxicity to the freshwater macrophyte Lemna minor exposed to uranium or to a mixture of uranium and cadmium.
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Due to anthropogenic activity uranium (U) and cadmium (Cd) concentrations have increased and they are present as co-contaminants in different environments. The objective of this work was to measure and model the influence of major ion and trace metal competition on the exposure of U, Cd and a mixture of both metals to the freshwater macrophyte *Lemna minor* L. The objective of this work was to measure and model the influence of major ion and trace metal competition on the exposure of U to the freshwater macrophyte *Lemna minor* L. Therefore the accumulation and toxicity to U in the presence of varying proton or cation concentrations (Ca^{2+}, Mg^{2+}, Na^{2+}, K^{+}) was measured using a standard 7-days growth inhibition test. From the experimental data complemented with speciation calculations (WHAM7) a Biotic Ligand Model (BLM) for U in *Lemna minor* was set up. In addition possible interactions between U and Cd were tested in a mixture experiment. These mixture experiments aimed at testing a U-Cd BLM. First a BLM based on accumulation of U into the plants was set up. Both a one and two site BLM-model was fitted for U, however, it was shown thatthe two-site model provided an inferior fit to either the constrained or unconstrained one-site models. Extension of the accumulation BLM for *L. minor* to toxic effects entailed prediction of accumulation for all exposures and fitting of a dose–response model to relate prediction and accumulation and effect. Toxicity was modelled based on the predictions of all three U accumulation models. It is shown that the two-site model provided the best fit, despite the fact that the corresponding accumulation model

gives the poorest fit. In the U-Cd mixture exposures it was shown that U accumulation is not affected by the presence of Cd in the mixture exposures. In contrast, there is a clear influence of U on the observed accumulation of Cd. In extending the two site BLM to U–Cd mixtures it was assumed that Cd^{2+} binding would occur only at one site. It could, however, be concluded that modelling of the interactions between U(VI) and Cd in combined exposures broadly suggested that competitive uptake is not the only mechanism required to explain the mixture toxicity patterns and that a better understanding of the mechanism of U(VI) toxicity to aquatic organisms is neededto provide robust predictions of uptake and toxicity of U(VI) in the presence of co–contaminants. Supported by the EC: STAR Fission-2010-3.5.1-269672

Advancements in life cycle impact assessment and footprint method development (II)

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Evaluation of Product Impacts on Biodiversity: The Product Biodiversity Footprint project
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Consumption of various products around the world is one of the main drivers of biodiversity loss. As biodiversity is an essential element of our natural capital, many indicators have been developed in the recent years to measure biodiversity degradation. However, few pertinent ecological indicators regarding product life cycle impacts on species and ecosystems are available at a product scale. In the Life cycle assessment (LCA) field, methodologies are improving to assess impacts on biodiversity, by already taking partially into account three of the five pressures defined in the Millennium Ecosystem Assessment (land use, pollutions, climate change). In order to reinforce the relevance of biodiversity loss assessment in LCA, the Product Biodiversity Footprint (PBF) project was launched in 2016 involving experts from LCA and ecological fields. The objective of the project is to develop a method and a tool crossing biodiversity studies and companies' data to quantify the impacts of a product on biodiversity. Coupling LCA and ecological data and methods is an emerging challenge to develop a reliable product biodiversity footprint. This approach is tested on three case studies in the project (food, textile and cosmetic industries). This project is a major step in helping companies to identify their impacts on biodiversity and to determine potential improvements.

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Forest Product LCA: Carbon Form, Fire, Fuel and Fate Rules
M. Vlieg, The Evah Intitute / Vlieg LCA; D.G. Jones, Ecquate Pty Ltd / The Evah Institute; S. Ashar, Global GreenTag
This work pushes beyond the limits of current life cycle methodology to enable modelling of potential and actual carbon sequestration and sinks. Whether LCA should account for carbon stored in forest product has long been discussed. It is argued that stored carbon should not be counted as it will re-enter the atmosphere sooner or later. But this time factor is critical as we approach tipping points in the next decades. Now many leading economists, Carbon Trusts, International and National Harvested Wood accounting frameworks, NGOs, National and State Government Policy, Manufacturers and Professions recognise the need for accounting of sequestered carbon. When LCIA practitioners currently often ignore carbon sequestration most significant legal and ethical issues arise. The paper proposes a new global approach for carbon capture and or loss in durable forest products considering the form of land management, the fire history of the forest, the fuel use on-site, and the fate of the product. The paper reviews >30 of the authors' cradle to grave case studies of plantation product supply chains. Data is from manufacturers of such as sawn lumber and laminated benches. LCI databases used for this work operate in global and local modelling and calculating engines including Boustead LCI Models. All emission factors are updated to 2013 IPCC. Specialist building and Simapro 8 software are used for ReCiPE, TRACI, and Eco-Indicator 99 Egalitarian ($EI99_{E10}$) Life Cycle Impact Assessment (LCIA) methods. IPCC 2013 CO_{2e} outcomes of forestry products were charted with and without carbon accounting. Results considered exclusion of Brazilian and Malaysian timber sequestration because of continuing loss of rainforest forms across both nations. Other products had either CO_{2e} uptake or sequestration as carbon sinks or a significantly lower CO_{2e} uptake. Results were reviewed for 60yr use of three forest products varying in carbon uptake and fuel use in processing. The significant difference in carbon uptake found depended on biofuel use for debarking, chipping, and sawmilling. Results were compared for timber fibre rich exterior cladding with various carbon sink values considering tropical interior boards and others. Forest product carbon sink values very significantly with management form, fire history, fuel use and interior and exterior applications and fate in re-use, recycling and landfill varied so LCA with detail knowledge of forest sources is essential.

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Is nitrogen considered properly in LCIA? Review on nitrogen assessment methodologies and their relevance for LCA.
K. Wowra, Technische Universitaet Darmstadt; L. Schebek, Technische Universitaet Darmstadt / Institute IWAR Material Flow Management and Resource Economy
Nitrogen is an essential nutrient for plant growth and therefore for the security of future food and feed production. However, the exceedance of nitrogen use has several negative impacts on the environment like eutrophication and acidification resulting in biodiversity loss. Nitrogen compounds have different implications on global or regional scale. In Life Cycle Assessment (LCA), these impacts are accounted for by assigning nitrogen elementary flows to the impact categories of 'climate change' or 'eutrophication', and 'acidification', respectively. Results of LCA studies have often shown trade-offs between these impact categories. These trade-offs result in the location of the impacts. Consequently, regionalisation should play an important role as to characterization factors of the impact categories eutrophication and acidification. However, the methodology of regional impact assessment as well as the relevance of regional environmental impacts of nitrogen compounds still are discussed controversially. To get a comprehensive overview on existing methods for nitrogen assessment, a review is carried out to evaluate nitrogen assessment in Life Cycle Impact Assessment (LCIA) and the consideration of environmental concepts to assess environmental impacts in LCA. Therefore, in a first step information on relevant LCIA methods are collected and evaluated regarding i) considered nitrogen flows in the impact categories acidification and eutrophication, ii) separation of environmental compartments iii) midpoint or endpoint level of characterisation factors and iv) regionalisation of characterization factors. In a second step a systematic review is carried out on environmental indicator concepts and their consideration in LCIA. In the last step, research gaps regarding the implementation of boundary concepts in LCA are identified. Seventeen LCIA methods were evaluated. Main differences could be identified in the considered regional scales and the analyzed nitrogen flows differed in regard to their contribution to the impact categories eutrophication and acidification. As to the systematic review on environmental indicator concepts in LCA seven recent studies could be identified dealing with implementation of environmental concepts as carrying capacity or planetary boundaries in LCA. Research gaps could be determined regarding the need of regionalisation of these concepts and the integration in LCIA methods.

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Market prices based valuation of abiotic resources in environmental assessment
S. Standaert, T. Huppertz, E. van Overbeke, B. De Caevel, RDC Environment
RDC Environment has developed a methodology based on life cycle thinking and monetisation to evaluate in a quantitative way the environmental, social and economic impacts of a product, service or policy. Monetising aims at reflecting the way human well-being is affected by an activity, with one euro meaning the additional welfare brought by one euro of additional income to a mean European (having a mean income). This paper focuses on the impact category "Non-renewable resource consumption". The cost for the society of consuming non-renewable resources is to be assessed. It corresponds to the well-being loss due to the increased resource scarcity in the future as a consequence of its consumption today. The valuation methods used in LCA are found unsatisfying by the recent reviews, while many CBA value abiotic resource assuming no issue of too fast depletion. This abstract is a development of the work presented at Setac Berlin in 2012 under the name "How to correct price for monetising non-renewable resource consumption?". Based on an analysis of the market failures affecting the market price, we observe that the main identifiable bias between market price and optimal price is due to the fact that private market agents weight future less than the world community would do, i.e. private discount rate is higher than societal discount rate. Hence, the market price is lower than the optimal social price and resources are overconsumed. We propose a method to correct for this bias using Hotelling's rule describing how resources price evolve with time according to the discount rate. The price of the last resource unit sold is extrapolated using a private discount rate. Then, the optimal price is retropolated with a social discount rate. The developments to the 2012 method regard (1) the distinction of the extraction cost and the net price, representing the "scarcity rent". The correction only applies on the net price and not on the extraction cost; (2) the taking into account of the demand elasticity: if the price is corrected, then fewer resources are consumed and the scarcity diminishes, leading to a lower equilibrium optimal price than estimated in the simple model of 2012 and (3) the use of time-decreasing discount rates instead of constant discount rates, better corresponding to economic literature and leading to lower price corrections. The method leads to proposals of monetisation factors and characterisation factors for non-renewable resource consumption.

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Poster spotlight: TU212, TU213, TU214, TU215

Bridging between ecology, ecotoxicology and ecosystems services (I)

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Bridging between ecology and ecotoxicology: what are suitable endpoints or response variables to detect effects on plants at different ecological levels?

E.M. Gross, University of Lorraine / Laboratoire Interdisciplinaire des Ecosystemes Continentaux; G. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment

Aquatic plants (or macrophytes) are important functional and structural elements of aquatic ecosystems. They convert solar energy and CO2 to organic matter, which in turn serves as a food resource, habitat and shelter for aquatic fauna. They are important for O2 production, nutrient cycling, and sediment stabilization, and thus key species for providing ecosystem services. Acknowledging this, they are explicitly considered in the risk assessment (RA) of contaminants. RA is based on data from simple laboratory tests including one test species, population- and ecosystem-level controlled tests in microcosm and mesocosm studies mimicking aquatic systems, and modelling approaches. Tests have to consider endpoints sensitive to the test compound(s). An endpoint can be defined as a variable reflecting plant performance and development during and after exposure to a toxic compound. Macrophyte and algae endpoints are usually sub-lethal. A range of endpoints might possibly be assessed, given the wide diversity in macrophyte growth form, morphology and physiology. Also physiological endpoints can be measurement endpoints. Not all measurement endpoints are appropriate endpoints, as toxicological sensitivity, variance and ecological relevance are important criteria to consider in the evaluation of the suitability of potential endpoints in plant toxicity experiments and RA. Moreover, endpoints need to be linked to the protection goals. At population and ecosystem level, macrophyte biomass is the endpoint assessed for evaluating effects, as no - or limited effects on biomass are a pre-requisite for sustainable plant populations in the field. However, physiological endpoints such as elemental composition, photosynthetic activity and the production of bioactive secondary metabolites potentially provide further insight into the performance of a given macrophyte in the interaction with competitors, herbivores or pathogens, thus at the community level. Plant biomass can be measured in experimental studies or predicted by models. At the field level, surrogate measures for plant biomass might be applied. At this level, eco-toxicologist might learn from ecologists in which endpoints are appropriate at different ecological levels (from populations to communities, ecosystems up to the landscape level) that can be used to assess plant performance and what level of effects can be considered as maximal acceptable for sustainable existence of plant populations and ecosystems.

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The performance of plant individuals, populations and species

M. van Kleunen, University of Konstanz / Department of Biology

Irrespective of whether one’s research focus is physiology, micro-evolution, community ecology or macro-ecology, all of us are ultimately interested in performance or organisms and variation therein. Indeed, most of the major questions in ecology and evolution require data on performance of individuals, populations or species. As these different levels of biological organization are nested, the performance of individuals contributes to the performance of populations, which in turn contributes to the performance of the species. Moreover, as organisms co-occur with others in communities, it are not only absolute measures but also relative measures of performance that are important for many of our research questions. In the ideal case, one would for an individual determine its life-time reproductive success, for a population its demographic growth rate, and for a species its spread rate. Unfortunately, these ideal measures of performance are frequently very difficult, if not impossible, to measure. Therefore, we can in most cases only measure proxies or components of performance. These performance proxies or components can nevertheless provide us with important insights. In this presentation, I will discuss performance measures and use examples from my own work on naturalized and invasive exotic plant species to show: (1) how plasticity in root morphology in response to spatial heterogeneity in nutrient availability increases biomass production of individual plants, and how this is associated with the invasion success of the species, (2) how establishment of populations of native, non-naturalized exotic and naturalized exotic species may changes with climate change, and (3) how a species ability to reproduce in the absence of suitable mates and pollinators determines its native range size and its global naturalization success. I hope afterwards to discuss with you how these approaches could be relevant for research in ecotoxicology.

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Measuring changes in aquatic ecosystems after disturbance by pollution or invasion of alien plants

I. Stiers, Vrije universiteit Brussel / Department of Biology

In an aquatic system, complex ecological interactions exist between native macrophytes and different biological communities in relation to trophic status, i.e. nutrient availability in the system. The structure of a macrophyte assemblage plays a key role in the structure and functioning of freshwater ecosystems by providing food, shelter and oxygen for other aquatic life. When an alien plant establishes in a freshwater ecosystem it will set up new interactions with the resident biota and hence it will become part of the food web. Invasive macrophytes are well known and often cursed ecosystem modifiers in the broadest sense of the word and recognized as the second major threat to global biodiversity. Ecologically, they modify the macrophyte community composition and species richness, deplete oxygen and alter the food web structure. The impacts that invasive alien aquatic plants have can be measured on all levels of biological organization: genes, individuals, populations, communities and ecosystems. Another major threat too many European aquatic systems is eutrophication with similar consequences as biological invasions i.e. decrease in species diversity, changes in the dominant biota and even development of anoxic conditions. Eutrophication has also enhanced the dispersal of invasive species in freshwater ecosystems and lends competitive advantage of invasive species relative to native species. Similar effects on changes in native and invasive macrophyte communities might occur under the pressure of anthropogenic pollutants. This presentation will provide an overview on how invasive aquatic plants change community structure and affect ecosystem processes, and how this can be measured both in the field and in indoor experiments. I will mainly focus on how invasive plants change native aquatic plant species richness, composition, relative growth rate and seed set and what the effect is of eutrophication on the performance of both native and invasive plants. From there I will point out perspectives on how these approaches can be used in a wider sense to include other stressors and disturbances, such as chronic or acute anthropogenic pollution.

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What can ecosystem services and resilience considerations add to pesticide regulations and the sustainability of agriculture?

A. Dollacker, Bayer Ag / Regulatory Policy; A. Raybould, Syngenta

Here we analyse how pesticide regulatory risk assessment and risk management is currently undertaken and whether applying ecosystem services approaches may alter the practical implementation of these activities. We also examine agricultural policies used for resilience-building, which is key to enhancing farmland biodiversity and the ecosystem services it underpins. We conclude that the main value of an ecosystem services approach in pesticide regulation is to integrate key principles, such as risk-benefit analysis, into risk management decisions. We argue that because risk assessments already seek to protect the species within ecosystems from which ecosystem services are derived, incorporating ecosystem services would not require extensive changes to current methods. This is particularly the case for the first screening level risk assessment methods (called lower tier) developed by regulatory authorities that focuses on effects on individuals of a species and uses representative species for ecotoxicology testing. Refinements of risk assessments can theoretically be envisaged at higher tiers through a reinforced use of functional assessment endpoints, if they would be available. However, as such endpoints are currently lacking for many services, the development of ecosystem models, field experiments or monitoring approaches that elucidate the relationship between changes in ecosystem structure, function and specific ecosystem services, would be required. Research to develop models and tests of ecosystem services therefore has potential value, but ought not to delay risk management decisions based on existing regulatory methods and processes. **In concert with the above, we advocate initiatives for resilience-building on agricultural <strong>land** and landscapes, rather than focusing on risk mitigation only. This approach is necessary to fully apply ecosystem services approaches and calls for the horizontal integration of coherent and complementary policies, regulations and initiatives across the agriculture and conservation sectors, which should include non-agricultural landscape planning.

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Panel discussion

Looking across organizational boundaries: exchanging ideas on mechanistic modelling between SETAC and the International Water Association (IWA) (II)

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Ecological modelling can link chemical exposure to effects on the population dynamics of aquatic invertebrate species for major European rivers

A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; H. Baveco, Wageningen Environmental Research; C. Lindim, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; I. Cousins, Stockholm University / Department of Environmental Science and Analytical Chemistry (ACES); P. van den Brink, Alterra and Wageningen University

Assessing the ecological quality of European water bodies in the scope of the Water Framework Directive (WFD) delivers important input for policy makers and water managers. One challenge in this respect is the ongoing difficulty to link the chemical and the ecological quality of water bodies. Besides attempts to utilise chemical and biological monitoring results, modelling provides another approach to meet this challenge. Such large scale modelling approaches for the assessment of the ecological quality of waterbodies in response to chemical exposure are intrinsically difficult to be assessed in great detail, because large temporal and spatial scales require simplicity in terms of habitat variability and ecological realism. This presentation will show results of ongoing modelling efforts in the EU

7th framework program SOLUTIONS project, where approaches for large scale integrated exposure and effect modelling are developed that will lead to overcoming the outlined challenges. In this approach, exposure dynamics for a number of WFD compounds are linked to parsimonious individual-based population models that account for lethal and sublethal effects on the population dynamics of a number of aquatic macroinvertebrates for the most important river catchments in Europe. The STREAM-EU model provides exposure concentration results at the level of subcatchments, that is at a scale of tenths of km^2. Links between exposure and effects are realised using linear or log-logistic dose-response relationships. Results are expected to deliver qualitative insight into impacts of chemical exposure on a choice of WFD relevant species and to enable the identification of more impacted regions for more than 30,000 subcatchments in Europe. Coupling the STREAM-EU and the ecological models appears as suitable solution for linking chemical and ecological status of water bodies in Europe.

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Modelling of population dynamics, food web and biostatistics
E. Billoir, LIEC - Université de Lorraine - CNRS / LIEC CNRS UMR

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ChimERA: integrating fate and effect modelling
K. Viaene, Arche consulting; A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; H. Baveco, Wageningen Environmental Research; P. van den Brink, Alterra and Wageningen University; M. Morselli, A. Di Guardo, University of Insubria / Department of Science and High Technology; F. De Laender, Université de Namur ASBL / Research Unit in Environmental and Evolutionary Ecology
Modelling is increasingly becoming more integrated in ecological risk assessments, both for exposure and effect assessment. Models allow for a more realistic assessment of the ecological risk but are not yet used to their full potential. The objectives of the ChimERA project were (i) to incorporate spatially and temporally heterogeneous exposure patterns, (ii) to assess how ecological interactions alter chemical effects and (iii) to develop a modelling framework that closely links chemical fate and effects models. To demonstrate the added benefit of this integrated approach, the ChimERA model was used to predict chemical effects in pond communities for 15 hypothetical scenarios, differing in the applied chemical, nutrient status, temperature and water flow. Both direct and indirect effects of these chemicals were predicted by the model corresponding to the sensitivities of the species. These effects were, however, heterogeneously distributed in space and time and reflected differences in exposure. These simulations demonstrate that the outcome of chemical exposure is determined by both the environmental and ecological conditions, which is difficult to capture with traditional risk assessment methods. Modelling tools like the ChimERA model can prove essential to answer current and future challenges for ecological risk assessment.

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Discussion

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Balancing complexity and uncertainty in model-based estimation of micropollutant fluxes in integrated urban drainage-wastewater systems
L. Vezzaro, DTU (Technical University of Denmark); P. Steen Mikkelsen, Technical University of Denmark / Department of Environmental Engineering
Integrated models of urban drainage-wastewater systems have been applied in the last two decades with main focus on reducing the environmental impacts (e.g. oxygen depletion, ammonia toxicity) from discharge of traditional pollutants. Model structures are usually defined with focus on computational requirements, since integrated models are utilized for long term simulations, scenario analyses, and optimization problems. In the examples based on the IWA models for wastewater treatment and river modelling, simplified descriptions of hydraulic dynamics and water quality processes are used. These approaches have successfully been applied in several cases. When dealing with micropollutants (MP), modellers are however faced by additional challenges: additional MP fate processes need to be included, dependency on other water quality parameters (e.g. particles and sediments) require more accurate models, and MP sources are not well described in existing models. Also, the need for a harmonic and parsimonious representation of MP fate processes across models of the different elements of the system (drainage network, treatment, recipient) is highlighted. While research activities focus on development of process models for single elements of the system (e.g. WWTP models describing different MP phases and byproducts), their extension to the remaining parts of the integrated systems is not straightforward and often neglected. Above all, a general lack of data on MP releases, concentrations, fate parameters and behaviour across integrated urban water systems strongly affect modelling results. Models combining approaches from chemical risk assessment with traditional integrated urban drainage-wastewater system models have been proposed, with a specific focus on fully exploiting the little available information. The first applications of these models highlighted how input uncertainty represents a major factor affecting the model results. Furthermore, integrated models are still facing challenges such as: (a) lack of measurements with sufficient time and spatial resolution to allow a validation of these dynamic models, (b) lack of knowledge and data linked to water quality processes taking place in the drainage network, and (c) lack of data needed by release models in urban areas. Generally, urban water modellers focusing on MP modelling should recognize the total result uncertainty and actively address it, similarly to what is done in chemical fate modelling at larger scales. We here provide examples of recent simulation examples illustrating these points.

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How certain are we about uncertainty of modelling approaches in environmental risk assessment?
T. Preuss, Bayer Ag / Environmental Safety; M. Wang, WSC Scientific GmbH / Dept Efate Modelling; A. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change; T. Strauss, Research Institute gaiac / gaiac - Research Institute for Ecosystem Analysis and Assessment
The consideration of uncertainty is an integral part of environmental risk assessment. While risk assessors may often not be fully aware of sources of uncertainties and how these are covered in a standard risk assessment which is performed according to agreed guidance documents and regulations, uncertainties are a more explicit component of mechanistic modelling. In general uncertainty is immanent in complex systems like environmental risk assessment. The uncertainty can either be addressed explicitly or more implicitly by application of (calibrated) assessment factors (e.g. using a NOEC from a standardised risk assessment with an assessment factor). The latter is widely used in established risk assessments, whereas modelling offers the tools to address uncertainty explicitly, e.g. by Monte-Carlos simulations or sensitivity analysis. Each step of a modelling approach has to be justified; therefore modellers explicitly specify the uncertainty of their parameters and sometimes also about the model structure. It is therefore important to clearly communicate not only about the uncertainty, but also the context in which this uncertainty becomes important. The uncertainty related to parameter estimation is of interest to anyone who wants to study this parameter, but might bear limited information about the predictive power of the model in a risk assessment context. In contrast, uncertainty related to model structure is of little interest for the scientists evaluating the parameters, but will increase the uncertainty of a model based risk assessment. In general uncertainty will be reduced in environmental risk assessment if variability within the system is explicitly addressed in different modelling scenarios and the current assessment factors (which include variability and uncertainty) are kept. Within this presentation we will discuss different approaches to address different types of uncertainties, namely parameter uncertainty, uncertainty of model structure and the uncertainty of model predictions. We will also discuss uncertainty of statistical and mechanistically model as well as the paradigm that more complex models are more uncertain, which is not necessarily true.

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Discussion

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Communication Approaches to implement mechanistic modeling in decision making
F. Verdonck, ARCHE
There are many mechanistic models to estimate exposure or effects of chemicals both in SETAC and IWA community. Some of these models are embedded in the decision-making process or even in the Regulation. Other, usually new, mechanistic models are yet to be implemented in decision-making. For this, models need to be credible and easy-to-use. This presentation will discuss two cases on how mechanistic modeling can be implemented in environmental policy and decision making at the regulatory level discussing achievements and difficulties. One case presents a communication approach based on simplifying a mechanistic, complex model to an user-friendly, easy-to-use model. The BLM (Biotic Ligand Model) predicts bio-availability of metals in surface water. However, they are data-demanding, time-consuming and insufficiently user-friendly. These drawbacks are significant barriers to the regulatory acceptance and implementation of BLM for routine use in EQS (Environmental Quality Standard) compliance setting under the Water Framework Directive and metals risk assessment in Europe and elsewhere. A simplified version of the BLM, named Biomet, was developed for less data-demanding and faster simulations at the cost of a small, acceptable decrease in precision compensated by an increase in conservatism. Biomet has been successfully implemented in national EQS compliance schemes. A second case aims at communicating complex models that are difficult to be described in a comprehensive, unambiguous and accessible way. A structured documentation CEN (European Committee for Standardisation) standard for large multimedia exposure models was developed to improve model applicability, transparency and credibility. The mechanistic model example using this standard is MERLIN-Expo. The documentation standard is based on existing model reporting formats but made

fit for science/research as well as for regulatory purposes by introducing several "end-user" specific levels of detail: the 'Basic knowledge' level of the structure targets towards 'basic' end-users who trust model developers on scientific, numerical and mathematical issues. The more advanced levels, 'Process knowledge' level (to better capture the scientific assumptions), 'Input data' level and finally, 'Mathematical knowledge' level, progressively targets end-user who wants to deeply understand the equations.

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Use of TK/TD models in pesticide risk assessment – Current situation and possible improvements
V. Poulsen, ANSES / French Agency for Food Environmental and Occupational Health and Safety
Risk assessment for pesticides used in agriculture addresses the risk for many no-target organisms. It is regulated by Regulation 1107/2009 that deals with data requirements and uniform principles. Additionally, SANCO and Efsa guidance documents provide all technical and scientific elements, and risk assessment schemes. They are all based on a step-wise approach, from basic worst case approaches to high-tier risk assessments based on more complex and more representative approaches. For example, in aquatic risk assessment, tier 1 risk assessment is based on single-species laboratory studies, and maximum PEC (Predicted Environmental Concentrations) values. Higher tier risk assessments can be based, for example, on modified exposure tests, which are intended to mimic more realistic exposure. These tests, that may be conducted with standard test species, are conducted to cover several exposure patterns. Experimental tests are indispensable, but can not be conducted indefinitely. TK/TD models, based on experimental data can be used to model biological reponses to these different exposure conditions. Currently, no model as been agreed and validated at EU levels. TK/TD models could become a very usefull tool for risk assessors in the future, but solutions have to be found. A a tool box, developped within academics, industry and regulators could be developped and widely used in a harmonised way for active substance monographs and/or for registration of plant protection products.

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Discussion and Forging Collaboration

Alternative approaches to animal testing for (eco)toxicity, and the regulatory application of the 3Rs in chemical risk assessments (III)

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How to use Fish Embryo Acute Toxicity (FET) Test Guideline (OECD 236) to fulfil the information requirements of REACH Regulation
A. Nyman, European Chemicals Agency (ECHA); M. Sobanska, European Chemical Agency; R. Cesnaites, S. Gutierrez Alonso, W. De Coen, European Chemicals Agency - ECHA
In July 2013 the OECD adopted the new Test Guideline for Fish Embryo Acute Toxicity (FET). The new OECD 236 test determines the acute toxicity of chemicals on embryonic fish as the LC50. The REACH Regulation (EC 1907/2006) improves the protection of human health and the environment from the risks that can be posed by chemicals. REACH promotes alternative methods for the hazard assessment. The prerequisite for use of data generated by alternative method is that it has to be equivalent to the results obtained by standard testing and adequate to draw overall conclusions with respect to the regulatory purposes as classification and labelling, PBT assessment and PNEC derivation. One of the standard information requirements for registering substances is acute toxicity to fish (AFT) (e.g. OECD TG 203). When new alternative methods are developed, it is important to consider if and how they can be used in the context of REACH. ECHA together with the external scientific consultant conducted such an analysis during 2015. The aim of the project was to assess the relevance and adequateness of using FET Test Guidance (OECD 236) to fulfil the information requirements and addressing concerns under REACH. In the light of the analysis made by ECHA, there are limitations in the use of the FET test guideline to fulfil the standard information requirements for REACH registration. The TG 236 would not be sufficient alone as a direct 'one-to-one' replacement for the AFT to meet the information requirement of REACH Annex VIII, 9.1.3, because there is currently inadequate evidence to establish clear applicability boundaries to decide under what circumstances the FET correlates satisfactorily with the AFT. Nevertheless, based on current knowledge, ECHA considers that the OECD TG 236 has a potential for use as part of a weight of evidence approach, in combination with other information, for the registrant to make a scientific justification to predict acute fish toxicity However, some of the limitations observed in the analysis may relate to limited amount of reliable and relevant data providing evidence that the FET test can predict adult fish toxicity to a certain class of substances. Therefore there is a need for further scientific investigations in order to use the FET test for regulatory purposes. In the current platform presentation will outline the outcome of the ECHA project and explore ideas on how the regulatory applicability of the FET might be enhanced.

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Benefits of Using Read-Across and In Silico Techniques to Fulfill Hazard Data Requirements for Chemical Categories
K. Stanton, F. Kruszewski, American Cleaning Institute
Substantial benefits are realized through the use of read-across and *in silico* techniques to fill data gaps for structurally similar substances. Considerable experience in applying these techniques was gained under two voluntary high production volume (HPV) chemical programs - the International Council of Chemical Associations' (ICCA) Cooperative Chemicals Assessment Programme (with the cooperation of the Organization of Economic Cooperation and Development) and the U.S. Environmental Protection Agency's HPV Challenge Program. These programs led to the compilation and public availability of baseline sets of health and environmental effects data for thousands of chemicals. The American Cleaning Institute's (ACI) contribution to these national and global efforts included the compilation of these datasets for 261 substances. Chemicals that have structural similarities are likely to have similar environmental fate, physical-chemical and (eco)toxicological properties, which was confirmed by examining available data from across the range of substances within categories of structurally similar HPV chemicals. These similarities allowed the utilization of read-across, trend analysis techniques and qualitative structure activity relationship ((Q)SAR) tools to fill data gaps. this presentation details the first quantification of actual benefits resulting from avoided testing through the use of these tools. In this evaluation of 261 substances, the use of 100,000 - 150,000 test animals and the expenditures of 50,000,000 to 70,000,000 (USD) were avoided.

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The Environmental Read-Across Assessment Framework for environmental hazard and fate endpoints
B. Versonnen, European Chemicals Agency - ECHA / Evaluation; D. Hirmann, European Chemicals Agency; A. Nyman, European Chemicals Agency (ECHA); A. Pirovano, M. Rasenberg, European Chemical Agency ECHA
REACH is a Regulation of the European Union [1], adopted to improve the protection of human health and the environment from the risks that can be posed by chemicals, while enhancing the competitiveness of the EU chemicals industry. It also promotes alternative methods for the information generation and hazard assessment of substances in order to reduce the number of unnecessary tests on animals. Grouping of substances and read-across is one of the most commonly used alternative approaches for filling data gaps in registration information packages submitted to ECHA under the REACH Regulation. This approach uses relevant information from analogous 'source' substances to predict the properties of 'target' substances. If the grouping and read-across approach is applied correctly, experimental testing can be reduced as not every substance within the group needs to be tested for each property. Grouping and read-across approaches have already been in use for a long time, but a general framework for assessing read-across approaches that would be applicable under REACH was not available. Therefore, ECHA developed a Read-Across Assessment Framework (RAAF) [2]. The framework to assess human health hazards was published in 2015 and the environmental hazard and fate part in early 2017. The RAAF is primarily designed for use by experts/regulators assessing read-across cases under REACH in order to facilitate consistency. The main principles and approaches are based on sound science and are therefore also very useful for scientists developing read-across cases and are not limited to cases developed under REACH but more widely applicable. The RAAF can be helpful when constructing well justified, scientifically underpinned grouping and read-across cases. In this presentation we will explain the main principles and approaches of the RAAF. Real-life examples will be presented to illustrate some of the common pitfalls and scientific flaws of read-across approaches, but also to illustrate how acceptable read-across predictions can be constructed.

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Ecological Threshold for Toxicological Concern (eco-TTC) - Assessing the potential of a new tool for environmental hazard assessment
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The Threshold for Toxicological Concern (TTC) is well-established for assessing human safety of indirect food-contact substances and has been reapplied for a

variety of endpoints including carcinogenicity, teratogenicity, and reproductive toxicity. Recently, we proposed an extension to the human safety TTC concept for application in environmental situations, termed the ecological TTC (eco-TTC). Eco-TTCs summarize the wealth of ecotoxicological information as Predicted No-Observed Effect Concentrations (PNECs) on diverse chemical substances in the form of statistical (probability) distributions. Eco-TTCs enable the prediction of untested chemicals based on structural attribute (category), mode of action, or functional use. The approach may be useful for assessing chemicals at early tiers of the risk assessment process, providing hazard perspective on chemicals that lack QSARs, guiding product development discussions, and assisting read across or category justifications. A database with approximately 110,000 unique ecotoxicological records has been developed based on recent assessments of published data and international chemical management programs. This toxicity data is associated with physical chemistry data and curated taxonomic information for the organisms tested. A process to conclude acute and chronic effects as well as identify the PNEC for exposed ecosystems based on depth and breadth of data have been devised and several mode of action schemes are being assessed to devise a best approach for grouping compounds. Chemicals that are categorized as neutral organics are the most abundant in the dataset, therefore are candidates for an initial in-depth assessment of eco-TTC attributes. Approximately 500 chemicals in the database are currently included in this analysis, with approximately one third having complete acute or chronic data sets (all three taxa). The eco-TTC for non-polar and polar narcotics is explored in depth, with additional categories under development (phenols, esters, reactive compounds, surfactants, pesticides, and pharmaceuticals). The dataset and associated tools will be made available via a web-based platform to provide an open, transparent opportunity for stakeholders to evaluate the approach with case examples. **The views, conclusions and recommendations expressed in this article are those of the author and do not necessarily represent views or policies of the European Commission or the U.S. Environmental Protection Agency.*

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ecodatahub - Big data techniques to create trusted, actionable ecotox data
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Recent advances in computer technology allow data collection and integration at speeds and in volumes greater than ever before, resulting in the “big data” revolution. The idea of big data presents an exciting new frontier, where large data stores promise problem-solving by virtue of volume and diversity. Yet important decisions are still made within the scientific community using only entity-level data: super-small compilations of trusted, specific data, typically stored on platforms like Microsoft Access or Excel. While these data may be highly vetted and reliable, they often lack the volume and density available from an integrated, multi-sourced dataset, and are difficult to access or integrate with other data sources. Evaluating the pros and cons of entity-level and big data approaches makes clear that a hybrid approach, which incorporates the best of both strategies to create actionable data, is ideal and feasible. Development of the hybrid approach will address one of the biggest limitations related to development of new, alternative methods: the lack of access to existing datasets that include the raw data needed to evaluate new methods. The ecodatahub proof-of-concept project will combine the reliability of entity-level approaches by using experts in the field to source data and determine data quality, while concurrently using big data techniques and data analytics to manage, integrate, and visualize data. The user-friendly ecodatahub interface will be free and publicly available so that users can easily interact with datasets and streams. The ecodatahub is supported by a multi-sector, multi-stakeholder group via financial and in-kind contributions, and is advised by a team of leading experts in toxicology, exposure science, chemistry, computer science, and risk assessment. This presentation will provide an overview of the hub strategy, proof-of-concept project, and future directions.

Interplay between nutritional factors and chemical toxicity

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Arsenic uptake by rice: Interactions with Silicon
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Rice (*Oryza sativa*) is a globally important food crop that is frequently contaminated by arsenic. Research has shown that silicon is an effective addition to minimize uptake of arsenite, but less is known about factors that minimize rice uptake of organic arsenic species, such as dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA). These species are the most commonly encountered organic As species in rice, particularly in rice grown in the southern USA. Silicic acid has also been hypothesized to play a role in uptake of DMA and MMA, but this has not been tested in long-term experiments. In hydroponic experiments, we exposed rice plants to either MMA or DMA and different concentrations of silicic acid throughout the life cycle of the crop. MMA exerted toxicity, regardless of silicon concentration, while DMA did not affect biomass. Silicon significantly affected DMA uptake, where increasing concentrations of silicic acid led to lower uptake of DMA in shoots, husk and grain. DMA also reduced yield, although this effect was alleviated by increasing silicon concentration. Notably, at the lowest silicon concentration, straighthead disorder was observed and very few grains matured. These data suggest DMA is a causal agent of straighthead, but the negative effects of DMA can be alleviated by silicon addition.

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Do fatty acids and methylmercury impact the function of adipose tissue in rainbow trout (Oncorhynchus mykiss)?
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Adipose tissue (AT) is an essential endocrine organ involved in energy homeostasis. Fatty acids (FA) are the major constituents of adipocytes as they form phospholipids in membranes and triacylglycerols in lipid droplets. In aquaculture, fish oil is increasingly replaced by plant-derived oil, which modifies the FA composition of the diet. Fish also face chemical contamination by heavy metals such as methylmercury (MeHg). Only few data are available on the influence of FA on rainbow trout AT and, to your knowledge, no study has investigated the effects of MeHg on adipocyte metabolism. Moreover, there is a poor knowledge of the potential interaction between FA and metals in fish. In this context, we used an *in vitro* approach with primary culture of trout adipocytes to assess the impact of (i) specific FA (eicosapentaenoic acid (EPA, 20:5 n-3), docosahexaenoic acid (DHA, 22:6 n-3), α-linolenic acid (ALA, 18:3 n-3), linoleic acid (LA, 18:2 n-6) and oleic acid (OA, 18:1 n-9)) and/or (ii) MeHg (0.5 to 10 mM) on cell differentiation and lipid accumulation. After 13 days of incubation, the supplemented FA represented the major FA in neutral lipids. OA and EPA also significantly increased the cellular lipid content, with a significantly higher effect for OA. Moreover, expression of lipoprotein lipase and fatty acid transport protein 1 were significantly higher in FA-enriched cells compared to control cells. The effects of other FA and MeHg are under investigation. An *in vivo* experiment was also carried out with rainbow trout juveniles (*Oncorhynchus mykiss*). It was divided in two phases: (i) an 4-week enrichment phase with a specific FA (EPA, DHA, ALA, LA) and (ii) a 6-week contamination phase during which MeHg was added to the diet at 0, 5 and 13.5 mg/kg of dry matter of diet. First results showed that fish fed with LA-rich diet presented significantly higher amount of total lipids than fish fed with EPA- and ALA-rich diets. MeHg showed no effect on lipid level. FA profile and expression of specific genes in AT are currently under investigation. In conclusion, we showed *in vitro* and *in vivo* that FA influence differently adipocyte lipid accumulation. FA also modified gene expression *in vitro*. Analyses of FA composition, gene expression and MeHg levels in AT of fish fed with FA-enriched diet contaminated or not with MeHg are under progress and will allow to better understand the effect of FA on fish AT metabolism and the potential interaction with MeHg toxicity.

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Lipids modulate cadmium toxicity in aquatic organisms
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Lipids are key nutritional factors affecting aquatic organism fitness and can therefore influence its ability to cope with stressors. For example, it has been shown that dietary polyunsaturated fatty acids (PUFAs) modulate cellular and individual response to chemicals. However, impact of PUFAs on cadmium (Cd) toxicity is poorly known. Still, we recently demonstrated that the phospholipid composition of rainbow trout liver cells (RTL-W1 cell line) is an important parameter influencing cell sensitivity to Cd. Indeed, an enrichment of the cell culture medium in specific PUFAs, i.e. alpha-linolenic acid (ALA, 18:3n-3) or eicosapentaenoic acid (EPA, 20:5n-3) significantly increased cell tolerance to an acute Cd stress while an enrichment in their n-6 counterparts, i.e. linoleic acid (LA, 18:2n-6) and arachidonic acid (AA, 20:4n-6) had no impact. The protective effect of specific PUFAs might have occurred through a decrease of metal cellular uptake due to modifications of membrane lipid composition and/or a modulation of intracellular parameters increasing the cell resistance to the damages induced by the metal. Thus, in a first step, we tested these hypotheses and demonstrated that the interaction between PUFAs and Cd acts, at least partly, through alteration of the expression level of specific genes involved in both fatty acid metabolism (e.g. de cytosolic phospholipase A2 (cPLA2)) and stress response (e.g. metallothionein B (MTB)). Indeed, the expression level of these two genes was lower in cells enriched in ALA, EPA and AA and challenged with 100 μM Cd than in unenriched cells challenged

with 100 μM Cd. This may suggest a lower breakdown of membrane lipids in cells enriched in the three latter PUFAs and a subsequent lower induction of eicosanoid synthesis by the Cd stress (for cPLA2), and a lower Cd uptake (for MTB). Both mechanisms could partly explain the protection induced by ALA and EPA. However, other mechanisms might be present as AA had no impact on cell sensitivity to Cd. Uptake and proteome analyses (in progress) will enable to better understand mechanisms of interactions. In a second step we aimed at validating our *in vitro* observations at a higher level of biological organization using both the large daphnid (*Daphnia magna*) and the rainbow trout (*Oncorhynchus mykiss*) as experimental models. Up to now, we validated the protective effect of EPA and ALA, previously observed in RTL-W1 cells, in adult and juvenile *Daphnia magna*, respectively.

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Do specific fatty acid enriched diets protect fish against Cadmium exposure and pathogen infection?

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Nutrition is crucial to grow healthy fish particularly in a context of pollution, overcrowding and pathogen risks faced in aquaculture. Since several years, the search for food components able to improve fish health is increasingly developing. Here, we investigated the influence of different polyunsaturated fatty acids (PUFAs) on the sensitivity of rainbow trout *Oncorhynchus mykiss* juveniles to Cadmium (Cd) exposure by focusing on immune responses. Fish were fed four diets enriched with one of the following PUFAs: alpha-linolenic acid (ALA), linoleic acid (LA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). After one month of preconditioning with PUFAs, the responses after a short (24 h) and a long-term (6 weeks) Cd exposure combined to nutritional trial were measured, and the resistance to pathogens was evaluated through a bacterial challenge with *Aeromonas salmonicida achromogenes* (week 10). The results showed that the diet enriched with DHA was more efficient in terms of growth performance than the LA diet, even after 6 weeks of exposure to Cd. The short-term exposure to Cd increased lysozyme and ACH50 activities for all diets except for ALA diet where ACH50 was decreased when compared to control LA diet. Moreover, the production of reactive oxygen species (ROS) in the kidney produced by different leukocyte populations was also modified by short-term Cd exposure in trout fed DHA and LA. However, after 6 weeks, the Cd did not affect any of the measured immune variables, even after bacterial challenge. However, ACH50 activity was decreased by long-term nutritional treatments with EPA. Our results suggest that supplementation with some n-3 PUFAs could provide several advantages in aquaculture, as it can improve growth performance (DHA diet), provide protective effect against oxidative stress induced by xenobiotic (ALA, EPA) and stimulate the non-specific immunity. Associated with previous findings from a study performed *in vitro* on liver cells, this experiment confirms the protective potential of some fatty acids with regards to the sensitivity of rainbow trout to Cd.

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The HERACLES Waste study: unraveling the associations between exposure to metals through diet and children cognitive functions

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Children are more vulnerable to environmental stressors than adults, and have less control over their environment. Although evidence indicating impact on children's development is growing, understanding of environmental risks to children remains still partial. In the framework of the HERACLES Waste study around 350 children aged 3 to 8 living in Greece in the proximity of the waste management plant (between 0.5 to 12 km) were enrolled to examine potential associations between exposure to metals through diet and modifiers thereof on their neurodevelopmental cognitive functions. Human biomonitoring samples were collected and analyzed for metals content (Cd, Hg, As, Pb, Mn and Hg). Four different test batteries were administered to children and their parents to assess potential neurophsycological and behavioral deficits in the study subjects. Moreover, socio-economic, demographic and frequency food questionnaires were filled in by the children parents in order to draw the picture of the lifestyle and more broadly of the socio-cultural context where the children live. An Environment-Wide Association Study (EWAS) approach was applied to assess the influence of exposure to metals through diet and exposure modifiers on children neurodevelopmental cognitive functions. Analysis of the influence of the children's diet shows interesting results, which corroborate the protective role of tomatoes (a food rich in lycopene, a natural antioxidant), and of foods rich omega 3 fatty acids (nuts, almonds, eggs) and the negative effect of pork and chicken meat or the dual effect of fish consumption. Among the exposure modifiers the distance from the waste treatment plant and the socio-economic-cultural conditions of the family showed a positive influence on the neurodevelopmental cognitive functions of children. The positive effect of these factors is associated to the better quality of life, translated as nutrition of higher quality, lower exposure to environmental contamination and better educational activities and opportunities. Overall, the results obtained support the utility of an EWAS approach to get a more accurate and holistic perspective to determine the association between population exposure and its determinants using human biomonitoring studies. A further step would be the analysis of the metabolome profile and metabolic pathway analysis which will allow us to further elucidate the effect of environmental and dietary components, as well as gender differences in response.

Marine and freshwater ecotoxicology (II)

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The importance of ecological processes and indirect effects in determining an aquatic ecosystem's response to ionising radiation

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The observed responses of an ecosystem due to radiation exposure depend on the different species sensitivities and the multitude of direct and indirect pathways by which individual organisms can be affected, including the potential for complex interactions across multiple trophic levels. Multi-species model ecosystems (cosms) were used to investigate effects of ionizing radiation on a model aquatic ecosystem, composed of both benthic and pelagic components, including indirect effects caused by ecological interactions. Microcosms were exposed for 22 days to a gradient of gamma radiation with 4 decreasing dose rates (20, 8, 2, 0.8 mGy/h). They consisted of two phytoplankton species (*Raphidocelis subcapitata* and *Eustigmatos* sp.), one zooplankton species (*Daphnia magna*), three plant species (*Lemna minor*, *Lysimachia nummularia* and *Egeria densa*), one gastropod species (*Lymnaea peregra*), sediment and litter bags containing dried leaves from four Nordic tree species. Several photosynthetic parameters (e.g. maximum PS II quantum yield (F_v/F_m) and apparent rate of photosynthesis (ETR) in all three aquatic plants were negatively affected by radiation in a dose-dependent manner. Non-photochemical quenching was highest in the higher dose treatments for all three plants species and ROS production increased with radiation dose in *L. minor*. Pelagic primary production decreased in all treatments during the first week and remained low for the duration of the experiment. Differences between treatments varied during the course of the experiment. This was probably due to the different timing in fluctuations in *D. magna* populations between treatments, in combination with potential direct effects to the phytoplankton. These temporal variations and treatment differences were also seen in measurements of whole ecosystem production and respiration. The benthic component show leaf litter degradation to be slower in all radiation treatments compared to the controls, with implications for nutrient release (C, N, P and major elements) to the sediment and water column. From previous cosm experiments we have shown the importance of changing nutrient concentrations and benthic-pelagic coupling in determining the effects of contaminants in aquatic ecosystems and we expect nutrient cycling to play a similar vital role in these systems.

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Impacts of radiation on DNA damage and reproduction in marine and freshwater amphipods

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Effects of environmentally relevant doses of radiation on non-human organisms are unclear owing to a lack of long term studies in contaminated environments. The renewal of interest in nuclear power as a low carbon energy source coupled with concern regarding past and potential nuclear incidents dictate that elucidating the impacts of radiation on the environment is of primary concern. Radiation exposure has been demonstrated to induce genotoxic and reproductive effects in a range of aquatic invertebrates. Studies have focused on female reproductive success and DNA damage in somatic cells in a limited range of organisms. The effects of radiation on male fertility has comparatively been ignored. This study aimed to provide a novel assessment of the impacts of radiation on male fertility and DNA damage in freshwater and marine amphipod species reflecting Chernobyl and Fukushima respectively. Males of *Echinogammarus marinus* and *Gammarus pulex* were exposed to phosphorus-32 (P-32) at a range of dose rates from 0, 0.1, 1 and 10 mGy/d. Uptake was assessed using liquid scintillation counting. The number and viability of spermatozoa was assessed using LIVE/DEAD sperm viability staining. The alkaline comet assay was performed on sperm cells following an adapted protocol. Individuals were paired with an unexposed female to assess knock on impacts on reproduction. Uptake of P-32 was five-fold higher in the freshwater *G. pulex* compared to the marine amphipod *E. marinus*. Radiation exposure led to subtle reductions in the percentage of viable sperm at all dose rates in both species relative to the control groups. Reductions were only significant at the 1mGy/d and 10 mGy/d dose rates in *E. marinus* owing to high inter-individual variability. No significant differences in spermatozoa numbers were recorded in either species. Reduced brood sizes were recorded in female *E. marinus* breeding with males exposed to 0.1, 1 and 10 mGy/d. An increase in the percentage of abnormal embryos in females breeding with males exposed to 1 and 10 mGy/d was recorded. Experiments are ongoing to assess the impacts of radiation exposure on genotoxicity in *E. marinus* and *G. pulex* and the consequences of a reduction in sperm viability in *G. pulex*. The finding that environmentally relevant doses of radiation detrimentally impact male fertility with potential knock-on effects to the next generation have implications for the assessment of risk posed by radioactive materials to the environment.

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Are invertebrate circulating cells inherently sensitive to oxidative DNA damage?
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Anthropogenic pollution is an ongoing concern in coastal management. The sensitivity of key species to DNA damage has been used to monitor genotoxic potential of environmental pollutants. Polycyclic aromatic hydrocarbons (PAHs) are amongst the most abundant pollutants in the marine environment with documented genotoxic potential. They interact with DNA and cause DNA damage, either directly by causing single- and double-strand breaks or indirectly through the generation of reactive oxygen species. The coelomic fluid of invertebrates acts as a transfer medium for pollutants, leaving circulating cells susceptible to deleterious effects. While single species have been shown to be susceptible to genotoxicity, a holistic view on species sensitivity is missing. This study determines the relative susceptibility of circulating cells of invertebrates from different phyla to DNA damage, following oxidative stress and PAH exposure. Circulating cells of mussel, sea star, crab and tunicate were exposed *ex vivo* to H_2O_2 (25 and 250 μM) and two model PAHs, phenanthrene and dibenzothiophene (0.5, 5 and 50 μM). DNA strand breaks, expressed as % tail intensity, were quantified using the comet assay. Circulating cells of the studied species show high sensitivity to oxidative stress with DNA damage exceeding 80% for most of the species and showing dose-dependency. Differences in DNA strand breaks between species following PAH exposure are likely attributed to differences in biotransformation efficacy of hydrocarbons. Our study showed that invertebrate circulating cells are highly sensitive to increased oxidative stress and react to PAH exposure. We showed that circulating cells from mussel, sea star crab and tunicate are suitable for early detection of genotoxic effects in the marine environment.

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Assessment of DNA integrity of Palaemonidae spermatozoa using the alkaline Comet assay: a pertinent tools for environmental biomonitoring
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The assessment and maintenance of aquatic ecosystem quality increase the need to developed robust biomonitoring tools as biomarkers in key estuarine and coastal species, and particularly in crustaceans, which has received less attention. In view of dramatic increase of man infertility in the past fifty years, contaminants induce alterations of semen quality in wildlife and its potential repercussion on population fitness can be legitimately addresses as a major issue in ecotoxicology [3-4]. In Crustaceans, in spite of an abundant literature relative to male fertility in aquaculture context, finally few studies were interested to effect of contaminants on sperm quality. Sperm of crustaceans displays cellular morphologies and functionalities (e.g. aflagellated and non-mobile, non-systematic occurrence of mitochondria and acrosome) [2] that are very differents from the more usual ent-aquasperm commonly found in other invertebrates as mollusks or in vertebrates. This apparent diversity limits the methodological transfer of some sperm quality markers classically used, as mobility, acrosomal membrane integrity or mitochondrial function. So genotoxic approaches seem to be a great research pathway to develop biomarkers of fertility on spermatozoa of crustaceans. Previously, the methodology and the sensitivity of the Comet assay with spermatozoa of Palaemonidae were tested. The next step of my PhD studies was to understand how interpret the level of DNA damage on spermatozoa of two species (i.e. the estuarine species (*Palaemon longirostris)* and the coastal species (Palaemon serratus)). In the first time, establishment of a baseline level was done (1) by monitoring spermatozoa of *P.serratus* for 2 years on a reference site and (2) by spermatozoa depuration of *P. longirostris* in the laboratory due to the difficulty to find a reference estuary. In a second time, we estimated the discriminated powerful of our biomarker in differentially impacted *Palaemon sp.* populations along the Estuary and the Bay of Seine for two years. The results suggest important DNA damages in populations from the Seine estuary and a lower effect in populations away from the estuary. In conclusion, this work showed the pertinence and the potential of the alkaline comet assay on Palaemonidae spermatozoa in the monitoring of environmental genotoxicity.

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Poster spotlight: TU040, TU041, TU042, TU068

Effects and ecological consequences of aquatic exposures to particulate materials from the nano- to macro- scale

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The dilemma of physical effects in aquatic toxicity tests
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A base set of freshwater aquatic toxicity tests using representative pelagic species are conducted to establish the environmental hazard potential of a chemical substance. Guidelines for conducting these tests were developed on the basis that waste water treatment infrastructure limit environmental aquatic exposures to substances as undissolved particulates. Thus, the presumed worst-case exposure considers the dissolved concentration as the major determinant for uptake and toxicity in aquatic tests. In practice many chemicals are sparingly water soluble and procedures must consider the need to remove excess undissolved material before exposure. Undissolved material present in the test solution has the potential to exert adverse effects on the test organism that are unrelated to intrinsic chemical toxicity, but rather due to physical interactions. The difficulties and similarities encountered with physical effects arising from exposure to undissolved material are illustrated using examples from aquatic testing of pesticides, suspended solids, nanomaterials and dispersed polymers. \nIn aquatic exposures the intrinsic toxic effects from the dissolved fraction are far greater than potential physical effects from undissolved materials. Aquatic exposures to particulates tend to be dynamic and are influenced by different exposure factors than the dissolved fraction. In an aquatic test with particulate material, exposure to the dissolved and particulate fractions occur simultaneously and the results do not isolate the physical hazard related to particulates. Thus physical effects from particulates are regarded as a confounding factor in aquatic toxicity testing. For toxicity testing to yield meaningful results it should be undertaken considering likely worst case exposure scenarios. Toxicity tests with pelagic organisms, are meaningful for particulate substances forming stable dispersions under environmentally relevant conditions. In most environmental scenarios, particulate exposures to pelagic organisms will be transient and localized, as inevitable aggregation/agglomeration processes will remove particulates from the water column. Generally, sediment toxicity tests may be more relevant worst-case exposure test systems to address both chemical and physical hazards from poorly soluble chemical substances rather than tests using pelagic aquatic organisms.

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An internationally benchmarked approach towards the standardised aquatic ecotoxicity testing of nanomaterials
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As part of the EU FP7 project NANoREG, we addressed the need for development or modification of exposure and (eco)toxicity methods to generate more reliable data for regulatory risk assessment of manufactured nanomaterials (MNMs). The key challenges identified were: (i) generation of reproducible MNM dispersions, (ii) measurement of dynamic MNM properties during standard aquatic ecotoxicity tests, and (iii) a lack of specificity in existing standard tests for MNMs. Using synthetic amorphous silica (NM-200) from the OECD Working Party on Manufactured Nanomaterials' sponsorship programme as a reference, we present a benchmarked standard operating procedure (SOP) for the calibration of probe sonicators that allows generation of comparable and reproducible dispersions. We also present a MNM dispersion SOP that is performance tested using 14 different reference materials representing metal, metal oxide and carbon nanotube (CNT) MNMs. A minimum of three data sets for each MNM were generated by different laboratories using in-house calibrated probe sonicators. Measurement of the resulting MNM dispersions is by simple dynamic light scattering (DLS). The results show that reproducible MNM dispersions can be generated, both with the same laboratory and across different laboratories, for all MNMs that are suitable to DLS (i.e. granular MNMs). There are limitations with the DLS analysis in the case of high aspect ratio MNMs such as CNTs and silver rods, which may also be linked to the stability of the dispersions. A technical guidance document (TGD) on procedures for the quantification of MNM exposure and fate in dispersions for aquatic ecotoxicological studies has also been developed. The TGD has the goal of identifying the minimum level of characterisation that allows meaningful

interpretation of ecotoxicity data generated. The TGD uses a decision tree approach for identifying how and when to determine changes in MNM particle size and morphology over time, nominal and total measured MNM concentrations over time, MNM dissolution and changes in dissolution over time, and the amount of MNM lost from the exposure due to deposition. The probe sonication and MNM dispersion SOPs, together with the quantification TGD, have been applied to the testing of one silver MNM (NM-300K), three TiO_2 MNMs (NM-100, NM-101 and NM-103), and three CNT MNMs (NM-400, NM-401 and NM-411) in optimised freshwater microalgae and *Daphnia magna* acute ecotoxicity studies.

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Short and long-term studies to ecotoxicological impacts of engineered nanoparticles in aquatic model systems
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The ecotoxicological effects of engineered nanoparticles (ENPs) are mostly unknown. However, their industrial use is continuously rising. Particularly, studies on prevalent and commercial relevant ENPs with innovative functionalized variations (e.g., coating or doping) are lacking. Therefore, the German Federal Ministry of Education and Research (BMBF) has launched the programme "NanoNature" in 2008. This programme supports and promotes research projects aiming to understand the impacts of ENPs on the environment in general and on organisms in particular. As a part of NanoNature "DENANA" ("Design criteria for sustainable nanomaterials") is a joint research project of 10 academic, industrial and regulatory partners that currently tries to conciliate the conflict of immediate marketing of innovative ENP-products and the precautionary principle regarding the potential environmental risks. Within this framework the Ecotoxicological Laboratory of the German Environment Agency (UBA) is investigating the biological effects of differently functionalized ENPs (silicon dioxide, cerium dioxide) on various aquatic organisms. Main objective is to provide a solid long-term data set on ENPs in aquatic ecosystems which can be used for hazard estimation and risk assessment in chemicals policy. Therefore, ecotoxicological tests with several species representing different trophic levels in aquatic ecosystems, ranging from green algae (*Desmodesmus subspicatus*) and higher macrophytes (*Lemna minor*) to invertebrates (*Daphnia magna*) and vertebrates (embryos of *Danio rerio*) were used. Moreover, multigenerational studies with daphnids were performed to estimate the long-term impact of ENPs. Additionally, several analytical methods such as dynamic light scattering (DLS) to describe the particle size distribution of ENPs, the determination of zeta potentials and chemical analysis of ENPs in aqueous solutions were conducted. Results will show which of the investigated ENPs cause ecotoxicological effects and whether functionalized variations are an appropriate criteria for designing sustainable nanomaterials.

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Exploring uptake and biodistribution of particles in biota
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In ecotoxicology, there is a continuous question if (nano)particle exposure results in particle uptake and subsequently biodistribution or that particles adsorb. To contribute to this understanding, we investigated the major factors that determine particle internalization into organs and within whole organisms. This is addressed by answering the following research questions: Is the exposure route important for uptake, and does the uptake route influence biodistribution? Literature search on all species and all (nano)particles of different chemical compositions was performed like also an experimental study with zebrafish embryos exposed to differently sized polystyrene particles. Following the literature study, no difference in uptake was found between oral and oral+dermal exposure routes. Our experimental study showed that particles were only taken up when exposed via the oral route, whereas the dermal route resulted in adsorption to the epidermis and gills only. In general, adsorption was found in all cases. Particles smaller than 50 nm are taken up by biota and are subsequent distributed within the organisms. Particles larger than 50 nm were predominantly adsorbed to the intestinal tract and outer epidermis of zebrafish embryos.

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Indirect and direct adverse effects of fine particulate matter on filter-feeding aquatic organisms
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The presence of synthetic nano- and microparticles in aquatic systems has initiated an increasing scientific and public interest on their occurrence and potential impact on human and environmental health. Up to date, there is no distinct scientific consideration of different types of particulates, e.g. natural vs. synthetic particles such as nanomaterials and microplastics. However, the dominant particle fraction in aquatic systems comprises inorganic fine sediments, which are considered a major stressor for stream ecosystems and their biodiversity. In particular, fine sediments have been suggested to play a crucial role in the declines of freshwater mussels, which are considered keystone fauna of streams and rivers. The effects of deposited fine sediments on recruitment failure of stream organisms are mostly explained by blocking the exchange between open and interstitial water. The effects of particles on adult mussels are less understood. The aim of this study is to provide an overview on indirect and direct stressor effects of fine particles on freshwater mussels. This includes the important functional role of streambed characteristics as well as mussel behaviour and mussel-dependent ecosystem services. Approaches for a representative characterization of streambed substrates, the determination of exchange rates between open water and interstitial and functional properties of the interstitial zone are presented. To test direct effects on individual mussel behavior, the mussel *Unio pictorum* was exposed to fine sediments of different particle size classes (< 45μm –125μm) and different concentration (0–10 gL^{-1}). Hall sensor technology and turbidity measurements were used to study mussel behaviour. Results revealed that the physicochemical properties of the streambed, especially substrate stability and the intensity of exchange between open water and the interstitial zone, are essential for mussel habitat functionality. Microparticles such as fine sediments showed to have a major influence on these habitat properties. As direct effects it could be shown that mussels improve clearance of suspended particles out of the water column by 35%, independent of particle size class and concentration, emphasizing their susceptibility to microparticle exposures. This study highlights that the role of inorganic particles should not be neglected since they represent the dominant particle fraction in the environment. Furthermore, the focus of future effect assessments should include both the effects of man-made and naturally occurring particles.

Applying Bioaccumulation Data to Better Inform Human and Ecological Risk Assessment of Chemicals

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Use of mammalian toxicokinetic data in bioaccumulation assessment
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Many substances are known to potentially bioaccumulate in air-breathing organisms. For such substances regulatory approaches to assess and generate further information on bioaccumulation in a systematic manner are not available and the need to develop a transparent approach has been repeatedly underlined by the regulatory and scientific communities. Mammalian elimination half-lives have been proposed as a potential complementary metric to assess bioaccumulative properties of a substance. Therefore, human/mammalian elimination half-lives and other toxicokinetic data have been compiled in order to explore the possibilities of using such data in bioaccumulation assessment. Respective data were collected on substances already identified as PBT/vPvB under REACH, as POP under the Stockholm Convention and on biocides, pesticides, veterinary medicines and PFASs with unknown bioaccumulation status. The data set currently consists of over 2000 elimination half-lives for over 1200 substances, supplementary toxicokinetic parameters, descriptions of test conditions and analytical methods, and information on substance identity. In addition, the data compilation has been supplemented with substance-specific physico-chemical parameters, such as logKow, logKoa, pKa and logD. The presentation will give an overview on the analysis of the data compilation and the results obtained and will discuss the findings from the perspective of assessment approach development.

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Development, testing and application of an in vitro to in vivo extrapolation approach for assessing biotransformation rates and bioaccumulation factors in fish and mammals: Lessons learned.
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To improve bioaccumulation assessments of commercial chemicals by (i) including methods for the assessment of biotransformation; (ii) extending bioaccumulation assessments to species other than fish; (iii) increasing through-put; and (iv) reducing costs and effort, we investigated in-vitro tests, in-vivo tests and in-vitro to in-vivo extrapolation (IVIVE) methods to assess the bioaccumulation metrics of hydrophobic, non-ionic organic substances. **Lesson 1**: The testing of in vitro substrate depletion bioassays using solvent delivery and passive dosing for a range PAHs showed a strong substrate concentration dependence of in vitro depletion rates following an apparent Michaelis-Menten kinetic profile. Bioassays using

passive dosing approximated concentration-independent (or maximum) biotransformation rates. **Lesson 2**: In-vivo experiments using a simplified dietary exposure design can provide somatic and gastro-intestinal biotransformation rates that can be used to test the IVIVE approach. Somatic and intestinal biotransformation rates are poorly correlated among the 85 chemicals tested, suggesting that one cannot be estimated from the other. Biotransformation rates appear to be highly dependent on the route of exposure. **Lesson 3**: In-vitro to in-vivo extrapolation (IVIVE) methods developed for pharmaceuticals can be simplified substantially for the purpose of assessing bioaccumulation metrics. This simplification provides an opportunity to expand bioaccumulation assessment to species other than fish as will be illustrated for mammals with the rat as the study species. IVIVE extrapolation tends to underestimate in-vivo biotransformation rates in all cases examined to date, but improves bioaccumulation assessments considerably. **Lesson 4**: Despite complexities associated with methodological complexities in vitro and in-vivo testing protocols, and uncertainty in the IVIVE modeling approach, some general "rules of thumb" emerge that may be useful for bioaccumulation assessment. With attention to the applicability domain, critical values can be identified for in-vitro depletion rates and in-vivo dietary uptake efficiencies that will cause BCFs and/or BMF to be below criteria values used in regulations. **Lesson 5**: There is a need to include in-vitro methods for intestinal biotransformation to further improve the application of the IVIVE approach for bioaccumulation assessment.

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Investigation of accumulation of persistent bioaccumulative toxic organic substances into passive samplers and aquatic organisms

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Pollution of surface waters by anthropogenic chemicals presents a significant threat to the normal functioning of aquatic ecosystems, in particular to aquatic organisms (biota). To assess the risk pollutant concentrations in water are measured and compared with regulatory quality standards or toxicological criteria, such as PNEC. However, water does not always seem to be the right monitoring matrix to evaluate environmental quality. Especially the group of hydrophobic organic substances (HOC) with low aqueous solubility are hardly present in the water phase and their concentrations in whole water samples mostly fluctuate in proportion to the the amounts of suspended solids. On the other hand, HOCs strongly accumulate in organisms and consequently, levels accumulated in biota are easier to measure for water quality monitoring. However, it is clearly a challenge to harmonize biota monitoring as concentrations in biota, even when collected from the same site, vary between species, among individuals from the same species, and with size, age, sex, physiological conditions and season. In monitoring programs already the species selection is problematic as it is not feasible to find everywhere the same species, even within a single river catchment. We took the challenge to investigate if and how HOC accumulation in elastomers, i.e. passive sampling, can be applied as a surrogate measure of biotic uptake. Passive samplers have constant properties; they are not affected by the variabilities listed above and can be applied everywhere. Passive sampling is generally associated with the measurement of (ultra-)low free dissolved concentrations in water, but in fact passive samplers are just an artificial matrix inserted and equilibrated with the surrounding environment. Chemical uptake to passive sampler can be interpreted by conversion to freely dissolved concentrations. Though, uptake can also be quantitatively converted to concentrations in other relevant matrices, provided their sorption properties can be quantitatively defined. For naturally occurring complex matrices like sediments and organisms this presents a major difficulty, which clarifies why freely dissolved concentrations – a phase with well-defined properties – are often useful in assessment of bioaccumulation. This presentation discusses the above views with examples demonstrating their practicalities. Preliminary results are shown of an investigation where extensive aqueous passive sampling is applied in parallel with sampling several fish species. The aim of the study is to investigate how passive sampling can provide an abiotic measure of bioaccumulated concentrations that may be comparable on a global scale. Acknowledgement: This work was supported by the Czech Science Foundation Grant No. GACR 15-16512S „Investigation of accumulation of persistent bioaccumulative toxic organic substances into aquatic organisms".

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Factors affecting bioaccumulation of cyclic volatile methyl siloxanes in a subarctic benthopelagic food web

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Cyclic volatile methylsiloxanes (cVMS) are used in personal care products and are emitted to aquatic environments through wastewater effluents. Due to the high hydrophobicity of cVMS, sediments are expected to be an important source for exposure in benthic and benthopelagic food webs. However, details of exposure pathways have yet to be fully understood. The main objective of this study was to evaluate the role of sediments in the bioaccumulation of cVMS, using a north-Norwegian lake (Lake Storvannet, 70 °N, 23 °E) as a case-study. Concentrations of cVMS were measured in benthos (chironomid larvae and the pea clam *Pisidium sp.*) and fish (three-spined sticklebacks (*Gasterosteus aculeatus*), arctic char (*Salvelinus alpinus)*, and brown trout (*Salmo trutta*)), while existing measurements of polychlorinated biphenyls (PCBs 101, 138, 153) were used to evaluate a new benthopelagic model. The aquatic module of the bioaccumulation model ACC-HUMAN was expanded to include a benthic filter feeder and a benthic predator/deposit feeder, and the model was parameterized for Storvannet. The pelagic version of the model over-predicted PCB concentrations in char and trout by factors of 3 to 16 compared to measurements (ratio between median values), and the benthopelagic model by factors of 2 to 7. Inclusion of the benthic link, therefore, improved predictions, particularly for char that feed mainly on benthos in this lake. D5 was the dominant cVMS congener in the lake, with measured concentrations of 2.0 ± 0.04, 17.8 ± 0.7, 3.2 ± 2.3, 3.8 ± 2.8, and 0.8 ± 0.6 μg/g lipid in chironomid larvae, *Pisidium* sp., three-spined sticklebacks, char, and trout, respectively. The benthopelagic model over-predicted cVMS concentrations in all species, but reproduced well the relationships between the different species. As Storvannet has temperatures around 0 °C for the majority of the year, the differing temperature-dependency of partitioning between organic phases and water for PCBs and cVMS was important for the model predictions. This was particularly pronounced in the benthic linkages within the model, where several differences in bioaccumulation behavior between PCBs and cVMS was observed. This highlights the need to increase the understanding of partitioning of cVMS at low temperatures. Also, it stresses the importance of understanding bioaccumulation behavior and biotransformation capacities in lower trophic levels, which are fundamental to understand also the higher trophic levels.

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Exposure assessment of aquatic environment in Adige river to flame retardants

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In recent decades, the interest for environmental issues has increased very quickly. Recently multimedia and aquatic food web models were applied to explore the fate and transport of flame retardants, including PBDEs, in many environmental matrices. In this context, the MERLIN-Expo tool was developed (FP7 EU project 4FUN) to provide an integrated assessment platform for environment, biota and humans, allowing the detection of scientific uncertainties at each step of the exposure process. The main scope of the present work was to assess the aquatic environmental exposure of Adige river to PBDEs by using the aquatic food web models (River Measurements, Invertebrate and Fish models) available in the MERLIN-Expo. The accumulation of PBDEs in fish species was simulated using concentrations in sediment cores and through the reconstruction of the real representative food chain. The aquatic food web models provided as output of the deterministic simulation the time trend of concentrations for twenty years of BDE-47 and total PBDEs in aquatic organisms included in the Adige river food web. For BDE-47 the highest accumulated concentrations were detected for two benthic species: *Thymallus Thymallus* and *Squalius Cephalus* whereas the lowest concentrations were obtained for the pelagic specie *Salmo Trutta Marmoratus*. The trend obtained for the total PBDE follows the one of BDE-47. The estimated concentrations in aquatic organisms were compared to the available measurement data for the three fish species sampled in Adige river. The obtained results show that the model underestimates of at least two order of magnitude the chemical concentrations with respect to the real values, due to the construction of real but not complete food web. For the total PBDEs the simulated results were more similar to the real data, as the difference between the real concentrations and the simulated ones were of one order of magnitude. The application of MERLIN tool demonstrated the feasibility to obtained a deterministic simulations of taget compounds for twenty years. Moreover, through the integration of the aquatic food web models with human exposure model, available in the MERLIN tool, a complete human exposure assessment can be achieved.

Assessment of PBT and vPvB chemicals: Requirements, challenges and policy implications

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Prioritizing and Assessing Organic Chemicals: Are PBT Criteria Still Useful Today?

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Prioritization of 23,000 substances on the Canadian Domestic Substances List (DSL) for further regulatory assessment, called Categorization, was completed in 2006. Ecological categorization was based on the regulatory criteria for persistence (P) and bioaccumulation (B) set out in the *Persistence and Bioaccumulation*

Regulations, and a chosen threshold for inherent toxicity (iT to non-human organisms). Categorization for human health priorities was based on the greatest potential for exposure to humans or as having health effects of concern based on classifications by other national or international agencies for carcinogenicity, genotoxicity, developmental toxicity or reproductive toxicity. Experience in Canada over the last 10 years has revealed that < 10% of organic substances captured using the ecological Categorization approach (i.e., PiT, BiT, and PBiT) during 1999-2006, resulted in a "CEPA Toxic" risk conclusion requiring further risk management in the first two phases of the CMP (2006-2012), including approximately 100 PBiT substances. Greater efficiency with prioritization using an *Integrated Approach to Testing and Assessment* (IATA) was thus sought for the third phase of the CMP in order to complete an assessment of the remaining DSL priorities by 2020. Over 600 substances were further evaluated using a new IATA-based Ecological Risk Classification (ERC) approach. It was hypothesized that the IATA driven ERC would better capture chemicals that matter when compared to a hazard criteria approach due to a high number of chemical descriptors used. When these two approaches were compared, dramatically different priorities emerge between them with the IATA approach yielding greater parity with human health priorities. The Canadian experience from Categorization and the first two phases of CMP has shown that the application of an approach based exclusively on criteria such as PBiT has its limitations for prioritization, primarily because it is highly susceptible to both false positive and false negative error for identifying chemicals that matter and from lack of direct consideration of exposure. Here we suggest that hazard criteria (e.g., PBT, vPvB) are best suited to identify concerns in the far field (e.g., contaminants of global concern) as a precautionary tool to account for the uncertainty associated with exposure quantification.

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What are bottlenecks in the current PBT assessment scheme and how can they be overcome?
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PBT assessments are intended to identify chemicals for which a risk assessment in terms of a PEC/PNEC ratio ("risk quotient") cannot be performed because they circulate in the global environment for long times and "safe" concentrations cannot be established for them. Here we investigate the current PBT assessment scheme with examples from two groups of chemicals: (i) non-polar hydrophobic substances, which often have "typical" PBT properties, and (ii) poly- and perfluorinated alkyl substances (PFASs), which are (extremely) persistent, but often not highly bioaccumulative. For several sets of chemicals from these two groups, we investigate the available chemical property data, in particular the values reported for the octanol-water partition coefficient (K_{ow}), bioconcentration factor (BCF), LC_{50} for aquatic organisms, and degradation half-lives. Many data for group-I chemicals are strongly affected by measurement problems that are most likely caused by the low water solubility and slow uptake by organisms of this type of chemicals. Many K_{ow} and BCF values are substantially too low, and many LC_{50} values are much too high. For PFASs (group II), the B and T dimensions are poorly documented, but the very high persistence of perfluoroalkyl acids is well established. PFASs with reactive end groups, such as fluorotelomer alcohols, generally form perfluoroalkyl acids in the environment. We recommend for group I that the B dimension be assessed in terms of bioaccumulation factors, BAF, because the BAF describes the B dimension of these chemicals better than the BCF, and that the T dimension be given low priority or altogether removed from the assessment scheme because for group-I chemicals the T dimension is covered by the B dimension (bioaccumulation implies baseline toxicity). For group II, we recommend that precursors of perfluoroalkyl acids are considered as P chemicals because of the extreme persistence of their tranformation products. Furthermore, the extreme persistence as it is found for PFASs is in itself is a source of concern because it implies irreversible exposure and, thereby, the potential for a wide range of adverse effects in humans and the environment. Chemicals with extremely high persistence should be assigned the same level of concern as acknowledged PBT chemicals.

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Hydrolysis and biotransformation-derived persistent and mobile organic contaminants potentially impacting raw and drinking waters
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Highly polar organic substances may be able to penetrate natural and artificial barriers and are thus mobile in the water cycle. If these mobile organic contaminants (MOCs) are persistent (PMOCs) against microbiological and chemical degradation, their removal during waste water treatment and drinking water purification may prove difficult. Since the most frequently used trace analytical method for the quantification of organic pollutants in aqueous matrices, reversed-phase high performance liquid chromatography - tandem mass spectrometry (RP-HPLC-MS/MS), is only of limited use for the analysis of very polar substances, little is known about PMOCs in the water cycle and only few have been extensively studied and monitored. PMOCs may be, among others, industrial chemicals, or transformation products thereof. Most transformation processes (e.g. hydrolysis and biotransformation) usually result in the formation of transformation products (TPs) with increasing polarity until either mineralization is achieved or a dead end TP is formed, thus potentially resulting in persistent and highly polar water contaminants. Many PMOCs derived from transformation processes may still be unknown and thus not be represented in suspect or target screening campaigns. As a consequence, only limited information about their occurrence and origin is available, which severely exacerbates the sophisticated monitoring and effective regulation of transformation-derived PMOCs and their precursors. In this study, we investigated hydrolysis and biotransformation of selected REACH chemicals which were expected to be potential PMOC precursors. The hydrolysis and biotransformation procedures were adopted from the respective OECD methods and analysis was performed with hydrophilic interaction liquid chromatography (HILIC) and, if necessary, RP-HPLC, both interfaced with an Orbitrap Velos Pro mass spectrometer. High resolution mass spectrometry (HRMS) and additional experimental data (e.g. H/D exchange) was utilized to elucidate the structure of detected TPs. The environmental relevance of (tentatively) identified TPs was assessed and used for prioritization of TPs for future monitoring.
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Persistence assessment of plant protection products (PPP) in the context of PBT evaluation in the EU
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The classification of a PPP as PBT is a cut-off trigger for EU registration under Regulation (EC) 1107/2009. If two out of three criteria are fulfilled the substance becomes a "Candidate for Substitution" which reduces the registration to seven years. Products containing these substances should be replaced on member state level after a comparative risk assessment. A working group of DG SANCO (now DG SANTE) developed a guidance for PBT assessment of PPP which was used to establish the list of Candidates for Substitution However, for the EU renewal program (AIR) EFSA also applies the ECHA guidance on PBT for PPP (Guidance on Information Requirements and Chemical Safety Assessment Chapter R.11: PBT/vPvB assessment) although this guidance is intended to be used for chemicals regulated under REACH. The guidances have a different focus since for PPP, which are data rich substances, many more environmental fate end endpoints are available than for chemicals under REACH. This also includes more realistic terrestrial field degradation or outdoor mesocosm studies. One of the main differences is the required study or reference temperature to which the half-lives should be normalised. The SANCO guidance uses 20 °C whereas 12 °C are required by the ECHA guidance. For water and sediment the SANCO guidance recommends to compare the $DegT50_{system}$ of the water-sediment study with the trigger for the degrading compartment which is the compartment where the substance partitions to due to its sorption properties. The ECHA guidance requires the use of the OECD 309 study for the P-assessment in water whenever it is technically feasible to conduct this study. More than 50% of the PPP substances will additionally become classified as persistent if the 12 °C reference temperature from ECHA guidance is applied for the P-assessment of PPP instead of the 20 °C required in the SANCO guidance. It can further be expected that the use of the OECD 309 as required by the ECHA guidance will characterise more than 84% of the substances as P in water. Exposure and risk assessment using relevant scenarios all over Europe can show that the underlying concerns resulting from persistence of PPP in different compartments are sufficiently addressed when applying the SANCO guidance.

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Methodologies for the assessment of environmental impacts of PBT substances for input to socio-economic assessments under the REACH Regulation.
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Authorisation in the EU REACH legislation aims to control the risks from substances of very high concern (SVHC). Authorisation is the process in which all uses of a SVHC (listed on Annex XIV of REACH) are banned beyond a specified date, unless approval is granted for continuing specific uses for a limited time. For SVHCs for which there are specific environmental concerns i.e. persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB), decision-making on the application for Authorisation is dependent on a socio-economic analysis (SEA), in which the applicants must demonstrate that the benefits from the continued use/s outweigh expected damage costs for society. An SEA for a PBT/vPvB requires estimation of the environmental damages (impacts) caused by the continued uses of the substance, which are being applied for. The estimation and quantification of environmental impacts are therefore key inputs to the SEA. However, the output of risk assessments on chemicals do not give estimates of impact, and other information on the specific environmental impacts of substances is often scant. Methodologies to more properly determine the amount

and concentration of PBT/vPvB substances in environmental compartments have been developed, such that the consequence of granted authorisation in terms of the environmental burden of a substance over time (and space) can be estimated. Understanding and quantification of environmental impact requires developing a concentration impact function. This then allows economic valuation of impact in a SEA. This paper presents research on two methodologies that attempt to enable the estimation of environmental impact, and to link that to what is required for a SEA to support a REACH Authorisation application for a PBT/vPvB substance. Method 1 attempts at building a 'concentration-impact' relationship, using available effects data, reporting of impacts in the environment and applying an 'adverse outcome pathway' approach. Method 2 builds an impact profile/score for substances based on P, B, T and LRTP properties, and quantifying impact cost based on abatement, removal and substitution costs. The methods are compared for their usefulness within the context of REACH SEA. This work builds on work previously presented at SETAC. Using hexabromocyclododecane (HBCDD) as an example substance, the possibilities and practicality of the methodologies are explored, along with the data needs.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective

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Challenges to implementing bioavailable environmental quality standards for metals in Northern Europe

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Surface water Environmental Quality Standards (EQS) in the EU have historically been measured as total or dissolved metal concentrations because the dissolved metal concentration has previously been considered as a good estimate of the bioavailable, hazardous fraction of a metal. However, the influence of water chemistry on the bioavailability and toxicity of metals, is now well acknowledged in the scientific literature. Accordingly, the EU has recently directed that the annual average EQS concentration values for nickel and lead in surface freshwater be set as bioavailable, rather than total dissolved concentrations. Also zinc, copper and manganese have bioavailable regulatory limits in use in some EU countries. Thus regulatory communities in the member states have to both familiarize themselves with the new concept and with the tools by which to convert measured dissolved metal exposures to bioavailable concentrations.. To this end, Biotic Ligand Models (BLMs) offer a practical and user-friendly tools for calculating the bioavailable metal concentrations. Although only few water quality parameters are needed along the dissolved metal concentrations Many regulatory jurisdictions have however not been measuring dissolved organic carbon and calcium concentration frequently enough and monitoring practices have to be changed. Although current BLMs have successfully been tested in most Central and South European surface waters, the Ca^{2+} concentration (in 75%), alkalinity (in ca. 30%) and pH (in more than 20%) about of Fennoscandinavian surface freshwaters are outside the validated range of a Biomet, one of the possible user-friendly BLM tools for a regulatory use. This difference in water chemistry in a significant fraction of Fennoscandinavian surface freshwaters compared to other European waters has raised reservations among environmental administrators on the utiity of current bioavailability tools in these waters. There is an urgent need for an assessment of the utility of BLM to set EQS for Nordic surface freshwaters. The environmental administrators in the Nordic countries are open minded to the BLM approach but are looking for further proofs of its utility in local conditions. The Nordic Council of Ministers has funded a project to produce chronic toxicity data for nickel, zinc and copper in surface waters outside the Biomet validation range. This presentation will report on an assessment of bioavailability in Fennoscandinavian freshwaters.

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Promoting "redox equality" in ecotoxicology: the case of Cr(VI) and Cr(III)

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Virtually any review on chromium toxicity includes in its introduction a statement along the line "Cr(VI) is much more toxic than Cr(III)" and concludes that risk assessment and management efforts should be particularly concerned with the environmental behavior and effects of Cr(VI). However, studies showing that Cr(III) has non negligible ecotoxicological effects are increasingly being published. We surmise that these contrasting findings can be reconciled by considering the strong dependence of Cr(III) effects on the composition of the exposure media. In particular, the composition of many exposure media used in ecotoxicological studies is such that the added Cr(III) rapidly undergoes hydrolysis, resulting in the formation of Cr oxyhydroxides ($Cr(OH)_3$) and, eventually, in Cr precipitation. As a consequence, when working with Cr(III), the actual exposure concentration for the test organisms can be much lower than that added at the beginning of the test. This phenomenon may lead to underestimate the toxicity of this form of Cr. To verify the relevance of these issues for the current hazard and risk assessment procedure for Cr(III), we examined the studies originally retained by the World Health Organization (WHO) to derive the Probable No Effect Concentration (PNEC) of Cr(III) to aquatic freshwater organisms. Additionally, we performed solubility calculations for Cr(III) in two standard ecotoxicological test media; one for algae and another for daphnids. The ranges of pH and water hardness for the exposure media used in the studies retained by the WHO were 6.75-8.0 and 20-200 mg/L (generally as $CaCO_3$, but sometimes unspecified), respectively. These values, in particular the circumneutral pH range, are known to favor the formation of insoluble Cr species, possibly leading to an underestimation of Cr(III) ecotoxicity. Our solubility calculations confirmed that Cr(III) concentrations added to ecotoxicological test media may exceed the corresponding solubility limits of Cr(III); which would also lead to the formation of insoluble chemical species and underestimation of Cr(III) ecotoxicity. Overall, the currently accepted ecotoxicological basis to support the consensus that Cr(VI) is more toxic than Cr(III) appear rather weak and the study of Cr(III) ecotoxicity should not be a priori dismissed on the grounds of its perceived low hazard and risk potential.

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Fate of an old mercury contamination: What are the natural background levels in sediments and benthic, marine organisms?

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Investigations in the 1980s showed elevated concentrations of Hg in sediment and biota along Harboøre Tange in Nissum Bredning, Jutland, Denmark. The contamination was caused by several types of earlier discharges - i.e. from the chemical factory Cheminova. We determined Hg concentrations in sediment profiles and biota at 10 stations in 2014. We interpreted the data according to OSPAR's Background Assessment Criteria (BAC) and Background Concentrations (BC), EU's Environmental Quality Standards (EQS) and the Norwegian Environment Classification System (NECS - used until recently by the Danish environmental authorities). Hg concentrations in the sediment had decreased considerably since the 1980s and all stations except for one had average Hg concentrations below BC and BAC (50 and 70 ng Hg/g dry weight [dw]) and were characterised as 'background' (< 150 ng Hg g^{-1} dw) according to NECS. Potential reasons for the reduction in the Hg levels in the sediments will be discussed. Average Hg concentrations in soft parts of blue mussels along Harboøre Tange were between 200 and 500 ng Hg/g dw, corresponding to 'moderately contaminated' according to the NECS and above both BC, BAC and EQS (50, 90 and 116 ng Hg/g, respectively). Periwinkles had average concentrations in the soft parts between 180 and 430 ng Hg/g dw - which are all below the NECS upper limit for 'background' (500 ng Hg/g dw) - but well above the EQS. Determination of Hg concentrations in sediment and biota in Danish coastal areas without any history of Hg contamination reveals that true background levels in some organisms (e.g. periwinkles) are somewhat lower (typically 50 to 100 ng Hg/g dw) than what is characterised as 'background' in NECS – and thereby in better accordance with the EQS. Sediment and mussel Hg concentrations have earlier been shown to be strongly correlated, and it is noteworthy that sediment concentrations in the previously contaminated area below the BC and BAC defined by OSPAR still lead to concentrations in mussels – and other invertebrates – exceeding the mussel BAC and EQS up to fivefold and more. Hg concentrations in other benthic organisms i.e. shrimps, cockles, eelgrass and seaweed from both the previously contaminated and reference sites will be presented. Conclusion: Even if sediment Hg concentrations have now generally decreased to be at or below OSPAR's BC, most of the invertebrates still exceed EU's EQS for mercury.\n

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How does the long-term aging in the soil change terrestrial ecotoxic impacts of anthropogenic metal emissions?

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Aging and weathering reactions of metals in soils (referred to as aging) can potentially change terrestrial ecotoxic impacts of metal emissions, but the knowledge about the reactivity of metals in long-term aged soils is scattered and inconsistent. As starting point for the inclusion of aging in ecotoxic impact assessment of cationic metals in soil, we carried out a literature review to determine the role of aging time on the solid-phase reactivity of Cd, Co, Cu, Ni, Pb, and Zn from various emission sources. The sources were classified as: spiked (with soluble salts), airborne, organic-related, mining and industrial waste, other antropogenic, and geogenic. Next, we developed a framework for inclusion of aging in calculation of comparative toxicity potentials (CTP) of metals in soils. In total, we retrieved 1090, 374, and 405 measured data points on metal reactivity for spiked, anthropogenic and geogenic contaminations, respectively, measured in 184, 278 and 234 different soils. Spiked soils had been aged by up to ca. 2 years, while anthropogenic sources by up to ca. 2 centuries. Average (geometric mean) reactive fractions and their variability were the largest (from 0.2 to 1 $kg_{reactive}/kg_{total}$) in soils spiked with readily soluble metal salts. They decresed with aging time. Average reactive fractions were somewhat smaller for antropogenic and geogenic sources, but their variability was also large (from ca. 0.05 to ca. 0.8 kg $kg_{reactive}/kg_{total}$). Apart from Cd, anthropogenic sources generally had higher reactive fraction as compared to geogenic metals forms, and we found statistically significant differences between reactive fraction of geogenic and various anthropogenic forms of Cd, Co, Cu and Zn when the covarying effects of soil pH or soil organic carbon were taken into account. Statistically significant differences in the reactive fraction between anthropogenic forms of Cd, Co, Cu and Zn were found occasionally. The influence of aging on reactivity of anthropogenic metals was not consistent and difficult to capture. Basing on these findings, we recommend including the influence of emission source and disregarding the effect of aging in toxic impact assessment of anthropogenic Cd, Co, Cu, Ni, Pb and Zn in soils.

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Highlights for soil health assessment using earthworms: in vivo, in vitro and molecular tools

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The potential risk of pollutants has been mainly studied in aquatic environments, while their effects in soils are poorly investigated despite the great complexity of soil matrix and the potential interactions of soil components with pollutants that could affect their toxicity. Different evaluation strategies have been proposed to assess soil health; among them the use of terrestrial sentinels is undeniable. Earthworms, key species in soil ecosystems, are one of the most studied sentinel taxa in terrestrial ecotoxicology and soil health assessment since their pollutant body burdens reflect environmentally bioavailable pollutant levels and can exert measurable responses and adverse effects. Among earthworms, *Eisenia fetida* is a model species for toxicological investigations that has been broadly used in standardized OECD toxicity tests to assess adverse effects regarding survival, reproduction rate and/or growth (OECD-207-, OECD-222). However, these approaches are limited when the amount of soils to be tested is high and when a rapid assessment is required. Therefore, there is a need to develop fast and accurate methodologies for rapid screening diagnosis of contaminated soils, understandable for decision makers. Biomarkers could give this indication of soil health affording integrative and comprehensive information avoiding misinterpretations. Complementarily, the potential of *in vitro* techniques and "omics" technologies is beginning to be harnessed to provide a number of promising applications in ecotoxicology. Hence, a strong effort is being presently carried out: (a) to perform extensive toxicity testing based on the assessment of biological effects caused by a variety of pollutants such as metal chlorides (Cd, Ni, Cu, Pb), nanoparticles (Ag NPs), and elutriates from real soils (vicinity of a closed and unsealed landfill, and in an abandoned mine area, both in the Basque Country) were used to expose coelomocytes in primary cultures, (b) to develop an efficient and cost-effective soil health assessment strategy (determine causes and consequences of pollution using both *in vivo* standard toxicity tests and innovative in vitro approaches together) that will provide straight forward advice and support to stakeholders involved in environmental protection. *Acknowledgements.* Basque Government (Consolidated Research Groups; IT810-13), University of the Basque Country (UFI 11/37) and Spanish Ministry of Economy and Competitiveness (Nanosilveromics Project).

Science communication and citizen science - strategies for successful stakeholder engagements

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Risk to What? Translating Environmental, Social and Economic Impacts of Remediation into Stakeholder Values

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The Portland Harbor Superfund Site, about 10 miles of the Willamette River in downtown Portland, Oregon, is contaminated by more than 100 years of agricultural, urban and industrial inputs, affecting residents, businesses, tribes, recreation and wildlife; there is considerable contention over remedial options. The Environmental Protection Agency (EPA) released a proposed plan for the Site on June 8, 2016. Regulatory decisions should consider affected communities' needs, and how these might be impacted; this requires that diverse stakeholders are able to engage in a transparent consideration of value trade-offs and of the distribution of risks and benefits of remedial actions and outcomes. We assessed the sustainability of a range of remedial options, including EPA's preferred option. The Sustainable Values Assessment (SVA) tool was developed to link environmental quality, economic viability and social equity metrics to a range of stakeholder values; metrics were scored and aggregated and options were ranked in terms of stakeholder group (SG) priorities. Stakeholder values were linked to the pillars of sustainability and also to a range of metrics of these values. Remedial options were scored for each metric, using data provided by the EPA and a range of standard and innovative approaches such as Net Environmental Benefit; Regional Economic Impact, footprint, GIS and stakeholder analyses; metric scores were aggregated to value scores. This provided a values-linked integration of option sustainability. In parallel, the views of >280 stakeholder groups were evaluated, documenting a diversity of priorities. The sensitivity and robustness of assessments to diverse priorities was assessed by weighing value scores in terms of SG priorities. To address environmental justice, a qualitative social effect distribution assessment was also carried out, evaluating who bears the costs, and who reaps the benefits of remedial options, in terms of demographics, space and time. This approach goes well beyond the CERCLA 9 criteria for evaluating remedial options and allows for the communication not only of traditional sustainability "scores" for remedial options, but also how options might be ranked given the values and priorities, as well as exposure or access to various risks and benefits, of different stakeholder and demographic groups. This approach identified trade-offs and points of contention, providing a systematic, transparent valuation tool for community engagement.

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Conceptualizing stakeholder engagement in the realm of tackling air pollution and nurturing environment-conscious citizens, in the context of sustainable, urban resilient cities

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In order to establish sustainable long-term policies for urban impact assessment in support of air quality and climate change, contributing to the improvement of health and life quality, the integration of political actors, citizens and stakeholders may result in significant social and economic benefits. This study focuses on the engagement of citizen communities in air pollution and environmental monitoring issues, based on the novel approach of transferring policy ownership to the society and thus reducing risk of failure. An encouraging policy is proposed for cities that encourage and trigger long-term changes in behavioural patterns and thus will nurture environment-conscious citizens, used as a refined tool for environmental health impact assessment. Integration of complex system dynamics will incorporate the interactions between activity sectors and the respective behavioral changes, under conditions of environmental and socioeconomic change (through agent-based modelling), enabling a novel as well as more interactive and engaging way of raising awareness on environmental issues. An engagement operational framework is proposed, through stakeholder consultation, capturing priorities that fit better to individual societal changes. Scientific partners, city partners and citizens work together with the aim to quantify the effects of implementing further policy measures and use the results to set up a strategy for healthy, smart and resilient cities with a legacy in the long term framework.

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Application of a Participative Methodology to Manage Multiple Pressures on Estuaries - Experiences with Stakeholder Communication in the Elbe Estuary

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The Elbe Estuary is characterized by a manifold and in parts contradictory utilization and high pressures to utilize resources. Shipping and harbor, agricultural land use, industry, recreation and tourism, flora-fauna-habitats and bird sanctuaries are the most important kinds of use. Multiple conflicts of interest, some of them with a long history, affect decision making and planning. Quality of life in the estuary in the context of future climatic changes, increased urbanization and industrialisation asks for long term planning. For the development of management strategies public support and participation are urgently needed. The present instruments for public participation in Germany were inefficient to guide the public participation for major projects like the next Elbe deepening, where public interventions stopped the building operations. To enable decision makers and public to assess the risks and develop management strategies and measures despite complexity and uncertain scientific information, risk communication and a deep understanding of the current relationships are needed. In the EU-funded research project ARCH – Architecture and Roadmap to Manage Multiple Pressures on Lagoons and Estuaries – a participative methodology was applied with the aim to form a common understanding of the complex situation in lagoons and estuaries. Inspired by the "European Awareness Scenario Workshops" a workshop methodology with a series of three workshops with scientists, stakeholders and decision makers was developed in the project. To ensure the participation of all relevant stakeholders a stakeholder analysis was carried out. Interviews were made before to identify current issues and to clarify to what extent scientific information and scientific experts are involved in the daily business and formation of opinion of the interview partners. Objective of the series of workshops was to develop a common understanding and a common "language" about the current status in the estuary, to create a common future vision for the estuary and to formulate a realistic strategy towards an sustainable estuary management. The workshop process helped the participants to gain understanding of relationships between environmental, social and economic processes and to develop a a relatively neutral perspective on the system. However, the challenge in the Elbe region apparently is not the provision of information but the unstructured way in which it is provided.

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What can Non-scientists Contribute to Marine Pollution Research?
J. Brant, Cefas; L. Gilliam, eXXpedition; J. Jambeck, University of Georgia; A. Kaerrman, MTM Research Centre, Orebro University; O. Lane, Biodiversity Research Institute; A. Barrows, Adventure Scientists; D. Papoulias, E. Penn, eXXpedition
Although 'citizens' have undertaken data collection for many years as volunteers, there is still apprehension from many scientists about the value that these data can provide. Concerns may exist about the quality of data collection (the importance of following standard protocols) or the distribution of 'effort' (more data from tourist areas) as well about whether important fundamental questions are being addressed or overlooked. *eXXpedition* is an example of a participatory science effort based on a 75' yacht, Sea Dragon, operated with an all-female crew. The research missions are thematically focused on microplastics and toxics in the environment and their influence on female health. This citizen science model uses a mixture of scientists and non-scientists, working together to gather otherwise difficult to obtain information about the marine environment on sailing voyages across the ocean and in the Caribbean. Water samples for a wide range of studies have been collected since their first Atlantic crossing in 2014 including: surface tow and bucket samples for plastics, samples for perfluorinated alkyl phenols, samples for eDNA of marine microorganisms, and samples to test for endocrine disrupting compounds. Additionally, the geographic coordinates and descriptions of marine macro waste and, abandoned fishing gear are recorded in NOAA's Marine Debris Tracker. Information on sighting of sea life and marine birds has also been requested by some researchers. A unique aspect of this citizen science program is that the women crew members are tested for body burdens of persistent organic pollutants in plasma and mercury in hair. These data are part of much larger international initiatives on demographic trends of these compounds and provide a unique conceptual link between environmental contamination and personal contaminant levels. This presentation will focus on the range and quality of samples and datasets collected and the challenges and opportunities when working with non-scientifically trained volunteers on a sailing vessel.

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The use of photography in science communication - Its potential and its challenges
A. Bonisoli Alquati, California State Polytechnic University, Pomona / Department of Biological Sciences
In our increasingly visual culture, communication through images has a greater potential than ever to engage the public and communicate information and concepts. While environmental journalism has long embraced the illustrative and evocative power of images to create engagement, the use of photography by scientists in the context of science communication is still in its infancy. Visual communication is often relatable, memorable, concise, and allows avoiding technical jargon. All these characteristics are generally praised by science communicators as effective ways to convey scientific results to non-scientists. In addition, images can be distributed without modification, allowing a message to faithfully spread. Yet, as promising as this may sound, communication through photography has its own peculiar challenges. Contrary to the common perception of photography as an objective way of capturing reality, photographs are the product of subjective choices, both technical and semantic. In addition, effective communication of science through images requires scientists to become adept not only at crafting photographs, but also at storytelling through images.

Advancing science and application of planetary boundaries and related ecological limits concepts to enable absolute sustainability assessments

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Dealing with variability in ecosystem vulnerability for environmental management
M. Zijp, RIVM / DMG; L. Posthuma, RIVM / Centre for Sustainability, Environment and Health; A. Schipper, PBL Netherlands Environmental Assessment Agency; C. Mulder, RIVM / Lab. Ecotoxicology; M. Huijbregts, Radboud University Nijmegen
Human impact on ecosystems depends not only on the amount of human pressures such as ecotoxicity, nutrient stress and habitat change, but also on the vulnerability of the receiving ecosystems, which in turn depends on location-specific environmental conditions and species assemblages. Neglecting variability in ecosystem vulnerability might lead to either under- or over-protection. However, ecosystem vulnerability is not yet routinely quantified, mainly due to a lack of operational methods. Here, we define and apply ecosystem vulnerability distributions (EVDs): an operational way to account for variability in ecosystem vulnerability at different spatial scales. The EVDs are derived from stressor-response relationships of location-specific species assemblages to stress in combination with an impact level that should not be transgressed. They describe the vulnerability of ecosystems in a whole region based on a selection of reference sites. The EVD is then compared with the distribution of prevailing environmental conditions in the larger region, which results in stressor identification and ranking at various spatial scales. Application of our approach to freshwater fish assemblages in Ohio surface waters showed considerable spatial variability in ecosystem vulnerability. Of the stressors considered, physical habitat characteristics and total nutrient loads ranked highest, followed by ecotoxicity. Deriving and using EVDs is an operational way to account for variation not only in the stressors (the drivers) - but also in the stressed (here: the ecosystems). Ecosystem vulnerability can be operationally defined for a region based on a small selection of locations within a region. It can serve as basis for well-informed decisions regarding boundaries in sustainability assessments and environmental management.

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One Planet Thinking: Towards companies that perform within the earth's regenerative capacity
A. Kerkhof, J. Scheepmaker, Ecofys; G. Meijer, E. De Boer, Eneco; K. Blok, Ecofys
The latest Living Planet Report of WWF shows that we used the regenerative capacity of 1.6 planets in 2012 at present consumption levels. This demonstrates the urgent need to act within the earth's regenerative capacity in short term to avoid abrupt environmental change. An increasing number of companies set targets to lower the impact of their activities on the environment, but such targets do not inform if the company performs within the limits of the earth's regenerative capacity. Inspired by the concept of planetary boundaries, which was introduced by Rockström et al. in 2009, Ecofys developed One Planet Thinking (OPT) together with Eneco, an energy utility in the Netherlands, with the aim to link corporate activities to global, regional and local boundaries. In our presentation, we will introduce the methodological framework developed in which we link midpoint indicators of Life Cycle Assessment (LCA) to scientifically established boundaries at global, regional and local level. The methodological framework consists of three steps: 1) quantify environmental impacts at product level, 2) define scientific boundaries at global, regional and local level and 3) set targets at sector and product level. We will describe the challenges of linking the midpoint indicators of LCA to scientific environmental boundaries, such as the different positions in the environmental cause-effect chain, spatial and temporal scales, and fair allocation of efforts. The methodological framework has been applied to Eneco to set targets for climate change and particulate matter. The methodological framework should be seen as a first step towards absolute boundary setting for companies.

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Absolute environmental sustainability assessment using new Planetary Boundaries based LCIA methodology: framework and a case study
M.W. Ryberg, Technical University of Denmark / Quantitative Sustainability Assessment Department of Management Engineering; M. Owsianiak, Technical University of Denmark; K. Richardson, University of Copenhagen; M. Hauschild, DTU Management Engineering / DTU Management Engineering
The Planetary Boundaries (PB) framework introduced the concept of a 'safe operating space' for humanity, and defined PBs which should be respected to keep Earth in a Holocene-like state. The interest in the PB-framework led to a call for inclusion of the PBs in Life-Cycle Assessment (LCA) to advance absolute sustainability assessments. Here, we present a framework for operationalizing Planetary Boundaries based absolute sustainability assessments with LCA. The development of a framework for Planetary Boundaries based absolute sustainability assessments involved three main activities. First, development of a Planetary Boundaries based LCIA methodology (PB-LCIA) where the Earth System processes in the PB-framework were used as impact categories and characterization factors (CFs) were expressed in the units of the control variables for the Earth System processes. Second, as the control variables in the PB-framework express environmental states, the input for the CFs should be a continuous flux. Hence, the life cycle inventory (LCI) was made to express flows from annual fulfilment of the functional unit. Third, to perform absolute sustainability assessments at other than global scale, the 'safe operating space' should be allocated to a scale which reflect the assessed activity. Hence, absolute sustainability assessments should assess whether a product exceeds the share of the safe operating space it has been allocated. The framework's applicability was shown in a case study on annual potato consumption in Denmark. An LCI with environmental flows from annual production of potatoes was fed into the PB-LCIA to give impact scores. The safe operating space was allocated based on Danish population relative to global population combined with average share of income a Danish consumer spend on potatoes. The results of the case study showed that production of potatoes for consumption in DK is not environmentally sustainable as impact scores exceeded the allocated safe operating space for 7 out of 9 impact categories. It was found that potato production was mainly problematic for climate change, freshwater use, biogeochemical flows and land-system change. The result of this work is a framework and method for conducting absolute sustainability assessments with LCA. The key added value is the new PB-LCIA methodology which allow for expressing impacts scores in the units of the control variable in the PB-framework and for relating impacts to the safe operating space.

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Requirements for developing planetary boundaries impact categories and application to freshwater use

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It has recently been suggested to create a planetary boundaries (PBs) LCIA methodology to allow 1) expressing LCA results in the language of planetary boundaries, and 2) absolute environmental sustainability assessments. This study uses a comparison of planetary boundaries studies with a recent guidance document for LCIA indicators to identify 8 requirements for developing PB impact categories. These requirements relate to spatial and temporal concerns, impact indicators, impact pathway modeling, planetary boundary values, aggregation of results across time and space, the calculation of entitlement to safe operating space (SOS) and uncertainty management. The first five requirements are then applied to develop a preliminary impact category for freshwater use, covering all types of blue water consumption. Spatially and temporally derived characterization factors (CFs) were developed to translate LCI results to area equivalents of mean monthly water flows, i.e. the average area required to (re)generate consumed water flows within a given spatial unit and month. In addition, Planetary Boundaries Factors (PBFs) were developed, which translates LCI results into a different (larger) area equivalent, considering that a share of (re)generated water must be reserved to sustain aquatic ecosystems, in accordance with the Environmental Water Requirements concept. All data for the development of the factors and their spatial and temporal resolutions were sourced from the LCIA model of the AWARE indicator. Monthly average PBFs (weighted according to Human Water Consumption) range 2 orders of magnitude from 10^{th} to 90^{th} percentile and tend to be high in North Africa, Australia and parts of central Asia. The ranking of PBFs across spatial units is somewhat similar to that of the CFs of AWARE, which may be explained from the mathmatical proximity of the two underlying LCIA models. The CFs and PBFs can be used to express impacts from freshwater use in the language of planetary boundaries, but to allow for absolute sustainability assessments, they must be complemented by aggregation, entitlement of SOS and uncertainty assessment to obtain a final result. This will be a focus of our ongoing work.

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Absolute sustainability assessment of product life cycles through calculation of carrying capacity entitlement at industry level

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Absolute sustainability assessment in LCA is emerging as a new research topic. It involves the integration of carrying capacity references in LCA and the calculation of how much carrying capacity studied products can be considered entitled to. The latter is the focus of this study, which 1) presents a method for calculating industry level carrying capacity entitlement for the Gross value added (GVA) and Grandfathering (GF) approach, and 2) demonstrates the method in a case study of cow milk and soy milk for climate change and freshwater use. The GVA approach entails that the entitlement of an industry should be proportional to its contribution to the economy, while the GF approach entails that an industry “inherits” its proportion of the total impact in a past reference year as its entitled proportion of future impacts. The LCIs of the case study were tentatively modelled using EXIOBASE V3. Characterization factors and carrying capacities for water use (blue water consumption) and climate change were based on the planetary boundaries framework. All other parameters required to calculate life cycle impacts and entitlements were sourced from EXIOBASE. Preliminary case study results show that 1L of cow milk has a much higher climate and water use impact than 1L of soy milk. However, for water use, the unsustainable part of impacts (exceeded carrying capacity aggregated across industries) was found to be higher for soy milk than for cow milk when following the GVA approach, due to the relatively high contribution to GVA of the industries in the cow milk life cycle. For the GF approach, the water use of both products appear 100% sustainable. However, the spatial (49 regions) and temporal (annual time steps) resolution of EXIOBASE V3 “hides” potential carrying capacity exceedances in individual watersheds and months. For climate change, less than 1% of life cycle emissions were found to be sustainable for both milk products and entitlement approaches, which is due to the chosen global carrying capacity being controlled by the rate of the geological carbon cycle. Our approach can be considered a first step towards absolute sustainability assessments of product life cycles that rely on granting carrying capacity entitlement at the industry level. The approach may be refined and complemented by assessments that grant a single carrying capacity entitlement to studied products, based on, e.g., an evaluation of their contribution to meeting human needs.

Bridging between ecology, ecotoxicology and ecosystems services (II)

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Ecosystem services from a regulatory/risk management prospective

K.M. Nienstedt, European Commission - DG SANCO / PPR

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Chemicals: Assessment of Risks to Ecosystem Services (CARES). Where are we now and where are we going?

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Chemicals may have positive or negative effects on human well-being. The challenge facing decision makers is how to balance the wellbeing benefits provided by the use of chemicals with the potential wellbeing costs due to habitat degradation and loss of ecosystem services. Assessing the risk of chemicals to ecosystem services needs the development of tools and approaches for: (1) identifying what needs to be protected and where it needs to be protected within a landscape context and (2) translating ecotoxicological exposure and effects information into risks for ecosystem service delivery. The CARES project brought together stakeholders from government, business and academia to develop a common understanding of the merits and feasibility of an ecosystem services approach to chemical risk assessment and the implications for implementation. The project was organised in three phases, each with a stakeholder workshop. Phase 1 assessed the current state of knowledge and identified key information gaps and challenges (e.g. environmental complexity, data requirements, limitations in current testing/assessment methods). The need for a tiered approach was identified: lower tier using exposure- and/or effect-based triggers based on conservative assumptions; higher tier using standard scenarios to account for temporal and spatial heterogeneity in use and exposure patterns, ecological communities and ecosystem functions. Phase 2 explored the use of novel approaches from ecology, ecotoxicology and ecological modelling to address key information gaps. Case studies were used to illustrate the development of environmental scenarios and the potential use of ecological models and trait-based approaches to address issues of ecological complexity and heterogeneity. Stakeholders identified and prioritized the research needed to effectively implement an ecosystem services approach into prospective and retrospective risk assessment. The top needs were: linking measurement endpoints to ecosystems services; mechanistic models, in particular ecological production functions; scenario development; integrated decision making framework for risk managers and risk assessors; reference values for key ecosystem services. Phase 3 explored how an ecosystem services approach could be implemented and considered the implications for regulatory risk assessment.

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Ecological production functions: key attributes and challenges of implementation

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Acceptance of the conceptual framework for incorporating Ecosystem Services (ES) into environmental risk assessment and management has been growing. Operationally, incorporation of ES into risk assessment and management requires models that describe the relationship between ecosystems and ecosystem services. These models, or Ecological Production Functions (EPFs), may range from qualitative to quantitative. Bruins et al. (2017, IEAM 13:52-61) suggested that EPFs will be most useful for environmental management decision making if they quantify ES outcomes, respond to ecosystem condition, respond to stressor magnitudes or management scenarios, reflect ecological complexity, rely on data with broad coverage, have performed well previously, are practical to use, and are open and transparent. The inherent variability among ecological settings and the wicked nature of most environmental management issues require that EPFs be considered anew with each project, especially if the magnitude of the issue warrants detailed assessment. To be effective, the assessment process needs to start with a robust conceptual model that can then be translated into operational or computational models that adequately capture the essence of the particular ecological setting. Typically, multiple EPFs are needed for a complete assessment. The current practice of focusing on one or two endpoints, (e.g.., earthworms or pollinators in pesticide studies) provide some useful information, but are seldom sufficient to evaluate the multitude of drivers that influence the delivery of ES. The narrow focus of most assessments represents the greatest impediment for wider use of EPFs and ultimately may undermine much of the interest in using ES to inform environmental management decision making.

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Ecosystem Services in Retrospective Risk Assessment, Remediation and Restoration: State of the Practice

S.E. Apitz, SEA Environmental Decisions Ltd

With advances in our understanding of ecosystem services (ES), recognition of the interconnectedness between ecology, environment, and human uses has prompted new considerations for evaluating and protecting ecosystems. This has led to the recognition that landscapes and aquatic systems must be managed not only to maintain good ecological status at the field or river reach scale but also to sustain the viability and sustainability of landscape and aquatic ES at the watershed scale. Although traditional ecological risk assessment (ERA) is a powerful tool for sectoral, single-issue regulation and management, it tends to be uni-directional; focusing on the likelihood of negative impacts. We manage landscapes and aquatic systems to optimize selected valued bundles of ecosystem services, but these systems are inter-related. An ES approach to natural resource management (NRM) can provide a framework for balancing the desirable and undesirable impacts of decisions on environmental quality, economic viability and social equity. ES assessment may provide more meaningful insights into environmental impacts and social costs of anthropogenic impacts, as well as the net benefits and trade-offs likely derived from different management options. This results in an expansion of the current risk-focused thinking behind ERA to consider a range of desirable and undesirable responses by different ecosystem endpoints (service-providing units or SPUs). An understanding of the responses of a range of relevant SPUs to past or proposed changes to biophysical conditions (e.g., a change in land use, a remedial action, etc.) over time, if applied in a spatially explicit manner, can inform the management of landscapes. Using such an approach, both desirable and undesirable impacts on SPUs can be quantified and valued, but such a broad approach focusing on trade-offs is not always consistent with specific regulatory contexts, which can require a clear separation between risk assessment and risk management decisions. There are growing calls for the application of ES-based frameworks for retrospective risk assessment, but case studies remain rare, though slowly increasing in number. More case studies focused on restoration and remediation decision making exist, but approaches are far from standardized. Although there are obstacles to the implementation of an ES approach in NRM, including unclear regulatory and policy frameworks and the paucity of useful ecological production functions, many tools are currently available. The state of the practice for ES in NRM decision making will be reviewed. The focus and approach of a range of case studies and their regulatory context will be discussed, with an eye to identifying strengths, weaknesses, gaps and a path forward.

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Panel discussion

21st century Ecotoxicology and Human toxicology: Applications and perspectives for the use of OMICs data (I)

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Zebrafish transcriptomes after chemical exposure - a quest for common grounds
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In (eco)toxicological research “omics” technologies are increasingly used and supposed to improve chemical regulatory assessments in the future [1]. Several studies have analyzed the transcriptome of the zebrafish embryo after chemical exposure [2], however, to our knowledge, results of ZFE toxicogenomics studies have not been aggregated or compared so far, which is important to get a better understanding of the so far isolated results. For instance in many studies it could not be clearly resolved, which of the observed effects were specific for the compound or compound class studied and which would be expected to be of a more general nature, e.g. related to the embryonic stage or a disturbance of global homeostasis. We suspect compounds to act via a range of different molecular mechanisms (e.g. target molecules), but we also expect effect cascades to converge into smaller and more general sets of toxicity pathways [3]. Thus, we aimed at the identification of genes or gene sets showing a general response towards chemical exposure in a meta-analysis. We compared 33 published microarray studies including exposures of the ZFE to 60 different chemical compounds (948 arrays in total) and aimed at identifying common transcriptional regulation across all experiments as well as across meaningful subgroups. We found that the analyzed transcriptome studies show great heterogeneity in experimental settings which is probably one cause for the also observed heterogeneity in gene regulation. However, in selected experimental subgroups we could identify common trends of gene regulation and connected them to different biological processes. The analysis reveals some methodological constraints of existing ZFE transcriptome studies but it also provides a starting point to substantially increase our understanding about molecular effects and stress responses in the developing zebrafish embryo using transcriptome studies. **References** [1] Marx-Stoelting P et al. 2015. Application of omics data in regulatory toxicology: report of an international BfR expert workshop. Arch. [2] Williams TD et al. 2014. The toxicological application of transcriptomics and epigenomics in zebrafish and other teleosts. Brief. Funct. Genomics 13:157–71 [3] Villeneuve DL et al. 2014. Adverse outcome pathway (AOP) development I: Strategies and principles. Toxicol. Sci. 142:312–320

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Transcriptomic approach in microalga and macrophyte in-situ exposed in river impacted by chlor-alkali plant effluents
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Water quality degradation due to low concentrations of pollutants is a worldwide problem, but risk evaluation of chronic pollution *in-situ* is still a challenge. The present study aimed to evaluate the potential of transcriptomic analyses in representative aquatic primary producers to assess the impact of environmental pollution *in-situ*: the microalga *Chlamydomonas reinhardtii* and the macrophyte *Elodea nuttallii* were exposed 2hin the Babeni reservoir of the Olt River impacted by chlor-alkali plant effluents resulting in increased concentrations of Hg and NaCl in water. The response at the transcriptomic level was huge, resulting in up to 5’485, and 8’700 dysregulated genes (DG) for the microalga and for the macrophyte exposed in the most contaminated site, respectively. Transcriptomic response was congruent with the concentrations of Hg and NaCl in water in the Babeni reservoir affected by chlor-alkali plant effluents. Genes involved in development, energy metabolism, lipid metabolism, nutrition and RedOx homeostasis were affected by the *in-situ* exposure of both organisms. In addition, *C. reinhardtii* was affected for genes involved in the cell motility, while *E. nuttallii* was impacted for those involved in the development of the cell wall. DG were in line with adverse outcome pathways and transcriptomic studies reported after exposure to high concentrations of Hg and NaCl under controlled conditions in the laboratory. Transcriptomic response provided a sensitive measurement of the exposure as well as hints on the tolerance mechanisms of environmental pollution, and is thus promising as a more sensitive and an early-warning tool to assess water quality degradation.

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Elucidating the toxicity of the flame retardant tris (2-butoxyethyl) phosphate (TBEP) by transcriptomics
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Tris (2-butoxyethyl) phosphate (TBEP) is a compound produced at high volume that is used as both a flame retardant and a plasticizer. It is used as an additive in materials and as such, has been shown to migrate out of these materials and contaminate immediate environments. It is continuously detected as the most abundant flame retardant in house dust, while also being detected as far as the Arctic highlighting its persistence. Worryingly, they have been detected in human serum, urine, mothers’ milk, and placenta. TBEP could therefore pose a risk to human health. Toxicological data are largely limited to model laboratory animals, with data for human models being scant and non-comprehensive. Given that the liver is a major target organ for TBEP exposure, we used an RNA sequencing approach to explore the toxic effects in the human liver hepatocellular carcinoma cell line, HepG2 at the molecular level. Cytotoxicity assays revealed limited toxicity, with TBEP inducing proliferation at lower concentrations. Treatment with cub-cytotoxic concentrations of TBEP indicated that at these lower concentrations, the increase in proliferation is accompanied by an increase in protein, DNA, and energy metabolism. The most altered differentially expressed genes also revealed effects of TBEP on wound healing. Other effects include effects on immune function, organ regeneration, angiogenesis, and cellular proliferation, all of which further support the alterations to the wound healing process. Finally, TBEP was shown to alter steroid hormone biosynthesis, thereby highlighting its potential effects on endocrine disruption. This is the first such study investigating toxicogenomics of TBEP in human models, and agrees with the adverse health effects seen in other organisms.

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Daphnia magna transcriptomic profiling of chemically induced starvation responses highlights new molecular fingerprints with regulatory potential.
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Life history shifts are a key point when studying the effects of chemicals on organisms. While growth and reproduction are common endpoints with regulatory impact, these traits are heavily influenced by the energetic status of the organism and their capacity to obtain and process food. This makes it of extreme importance the understanding of the feeding capabilities of the organism. Chemicals which have the capacity of inhibiting feeding will also have a high potential to cause changes in population in a non-specific way. In this direction, is paramount to understand the potential impact that substances can have on feeding and what are the molecular specific fingerprints of exposure. In this work we exposed *Daphnia*

magna to six contaminants of different chemical properties (Cadmium, Copper , Cyalothrin, Fluoranthrene, Lindane and microcystins from live *Planktothrix* cells and starvation controls). Two feeding inhibition causing concentrations (aprox 20% and 60% inhibition) were used and a full transcriptomic characterization of the molecular patterns, using a recently developed Daphnia microarray was made in to understand the chemical and effect specific patterns. This profiling can have a high impact on how we understand the specificity of chemical exposure and the common pathways affected, giving us a much needed insight on the molecular and physiological cascade of events leading to chemically induced starvation responses with impact on populations and ecosystems.

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Prediction of genes involved in silver toxicity in Chlamydomonas reinhardtii based on gene co-expression networks
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Although the toxicological processes induced by some chemical stressors have been characterized, toxicity pathways and mechanisms of action at the cellular level are still largely unknown. Integrative bioinformatics analyses of large and diverse collections of omics datasets have the potential to reveal the mechanisms of cellular response to chemical stressors. Specifically, gene co-expression network analyses, which are based on the guilt-by-association principle, can identify hubs and modules of genes with similar expression patterns, indicative for structured, modular toxicity response characteristic to certain chemical stressors. We built a co-expression network of *Chlamydomonas reinhardtii,* based on 650 transcriptome samples taken from public databases, including an in house transcriptome study of silver toxicity. While the mechanisms of silver uptake/transport/export are mostly unknown, they have been linked to copper homeostasis. We searched the co-expression network around known copper transporters for genes that are differentially expressed under silver exposure, or genes that connect copper transporters to copper responsive transcription factors, and produced a list of genes potentially involved in silver toxicity. The respective *Chlamydomonas* mutants were then exposed to silver and ca. 50% showed a significant change in silver toxicity compared to the wild type, among them CTR2, a copper transported that has been hypothesized to be the source of silver uptake in green algae.

Behavioural ecotoxicology: Unravelling behavioural responses to aid environmental and regulatory toxicology (I)

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Dietary exposure to selenomethionine impairs learning ability in zebrafish (Danio rerio)
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A broad range of factors is known to influence cognitive ability in animals. However, the problem becomes more serious and complex when elements/compounds, which are vital for normal physiological wellbeing, become toxic. Selenium (Se), although an essential micronutrient, has been found to be a potential neurotoxicant during prolonged overexposures. Dysfunctions of neurotransmitter systems is one of the pathways by which selenium exerts its neurotoxic effects on the central nervous system. Dopamine is the most important catecholaminergic neurotransmitter in the vertebrate brain, which regulates a wide array of cognitive functions including learning and memory. In the present study, zebrafish (Danio rerio) was used as a model to investigate adverse effects of Se on learning and memory with possible involvement of dopaminergic system. To this end, adult zebrafish were exposed to one of five different concentrations of dietary L-selenomethionine (SeMet) (control, 3, 10, 30 or 60 µg/g dry mass) for 30 days. Learning performance was tested in individuals using a latent learning paradigm. Low levels of SeMet (3 and 10 µg/g) exposure did not significantly affect learning performance in zebrafish. However, fish treated with higher SeMet doses (30 and 60 µg/g) exhibited impaired performance in the latent learning task. The mRNA expression of dopamine receptors in the brain (telencephalon) was also differentially affected in different groups treated with high SeMet. In addition, high SeMet exposure resulted in a significant decrease in reduced to oxidized thiol ratio as well as an increase in lipid peroxidation relative to that in the control – indicating SeMet-induced oxidative damage in zebrafish brain. The findings of our study suggest that a deficit in dopaminergic transmission, through enhanced oxidative stress in brain, may contribute to behavioral and cognitive effects of Se exposure in fish. Keywords: Selenium; Latent Learning; Dopamine; Zebrafish

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ENVIRONMENTAL LEVELS OF ANXIOLYTIC PHARMACEUTICALS ALTER MIGRATION OF ATLANTIC SALMON IN BOTH LAB AND FIELD
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Humans consume more pharmaceuticals than ever and consumption is set to rise. As a consequence, increasing amounts of pharmaceuticals are released into waterways worldwide with virtually no knowledge of how they might affect aquatic ecosystems. Some conspicuous effects of these *emerging contaminants* are already evident including the feminization of fish by contraceptive residue. However, recent work suggests that important effects of pharmaceuticals in aquatic environments are much more widespread than currently believed, and that these effects may result in major changes in species interactions, population survival and ecosystem functioning. In several earlier laboratory studies, we have shown that concentrations of pharmaceuticals presently found in waterways alter important behavioural traits in both aquatic macroinvertebrates and fish, and that this in turn affects both feeding efficiency and predation risk. These results suggest that pharmaceutical contamination of aquatic environments may change species interactions, in particular predator-prey interactions, with severe ecosystem-effects as potential consequence. Recently our research focus has turned towards realistic large-scale studies in lakes and rivers using acoustic telemetry to test if findings from the lab also hold in natural settings. Here I present results from one such study comparing effects of environmental levels of the anxiolytic pharmaceutical Oxazepam on migration pattern of Atlantic salmon (Salmo salar) in the lab and the field. In the lab, salmon exposed to the drug migrated approximately twice as fast as unexposed salmon and the subsequent field-study generated similar results, validating the results found in the lab. This pharmaceutically induced change in migration-intensity has the potential to be a key determinant between survival and mortality of salmon individuals and as such important for population persistence as migration intensity is believed to be adapted to the environmental conditions of the river in question. The overall finding of recent studies suggests that effects of pharmaceutical contamination of natural systems might be much more widespread than we predict based on conventional ecotoxicological tests.

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Developing high-throughput, multi-endpoint, behavioural assays for model crustaceans
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Behaviours have been shown to be a sensitive endpoint in ecotoxicological testing, providing a link between biochemical and population level effects of pollution. Behavioural assays are generally faster than those looking at endpoints associated with growth or reproduction, and effects on behaviour have been found to drive down 'effect concentrations'. Despite this, endpoints associated with lethality, growth and development still dominate regulatory ecotoxicological assessments which is partly due to the lack of control data on behaviours of model species, furthermore differences in experimental design between studies have made it difficult to differentiate between closely related endpoints. For example, negative phototaxis can be interpreted as scototaxis and positive phototaxis can be interpreted as anxiety-like behaviours, resulting in a call for carefully designed and optimised experiments. Recent advances in computational equipment has facilitated the use of behaviour for high-throughput analysis of anthropogenic compounds. Most of this work has been done from a pharmacological discipline on vertebrates and is yet to be optimised and standardised for invertebrate species. Part of this ongoing study is to develop standardised, high-throughput behavioural assays for a range of model crustaceans to be used in ecotoxicological testing. Control data for three behavioural endpoints associated with zone use, preference for light or dark environments and swimming speed were collected for model crustaceans from both freshwater and marine environments. Studies were done on the amphipods *Echinogammarus marinus* and *Gammarus pulex* to optimise well shape and size for behavioural assays and to compare photo-sensitivity between species and individuals. Both species exhibited negative phototaxis and strong thigmotactic behaviour, spending >60% of time in the dark and >90% of time against a wall when placed in novel chambers. Swimming speed increased when amphipods were exposed to light compared to the dark as an avoidance response. Assays were then validated using the SSRI fluoxetine as a model compound. Work is currently being translated to *Artemia* and *Daphnia* with a view to develop more high- throughput screening of behavioural modulating compounds. This work demonstrates that velocity and phototaxis are viable endpoints for behavioural assays and that care must be taken in experimental design to distinguish between endpoints and understand behavioural responses.

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Male starlings fail to take a fancy to fluoxetine females
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Fluoxetine is a widely prescribed selective serotonin reuptake inhibitor (SSRI) antidepressant which is used to treat conditions such as depression and obsessive-compulsive disorder. One major pathway by which fluoxetine enters the environment is via sewage, since the parent compound is incompletely metabolised in humans. Fluoxetine has been detected in both solid and liquid fractions at waste water treatment plants (WWTPs) and in biosolids used to fertilise crop fields. Since

some wild bird species forage on invertebrates at WWTPs, they are at risk of exposure to fluoxetine. One relatively common side effect of fluoxetine in humans is sexual dysfunction. Many receptors which are targeted by pharmaceuticals are evolutionarily conserved across vertebrates. If effects similar to sexual dysfunction occur in exposed wild birds, courtship could be affected. In order to investigate the effects of fluoxetine on avian courtship, we conducted an *in vivo* chronic effects study in which we exposed wild caught Eurasian starling (*Sturnus vulgaris*) to an environmentally relevant concentration of fluoxetine. To measure the effects of exposure, we focused on a combination of behavioural and physiological traits linked with courtship. We found that compared with control females, fluoxetine treated females: 1) developed breeding colouration at a faster rate than control females, 2) were sung to less frequently and for less total time by males. However we found no effect of male treatment on male courtship behaviours and breeding colouration. We are currently exploring hormonal mechanisms for these effects, specifically circulating testosterone (T) and estradiol (E2) levels. If fluoxetine does alter courtship behaviour and physiology, it could have implications for breeding success in wild birds and may be a factor in observed population declines.

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Arbitrary experimental methodologies are influencing study outcomes in behavioural toxicity testing
S.D. Melvin, M. Duvignacq, M. Petit, Griffith University / Australian Rivers Institute
Behavioural endpoints are being increasingly applied in aquatic toxicity testing. These approaches are now widely accessible due to commercially available software and hardware options, and are indeed extremely valuable in many respects. For example, behavioural tests are generally recognised as offering increased speed and sensitivity compared to traditional toxicity tests (e.g., development and reproduction). However, criticisms have been raised about the relevance of non-traditional behavioural endpoints, and in particular the repeatability and scientific quality of many behavioural studies has been questioned. One of the most notable subjective aspects of behavioural toxicity testing relates to the highly arbitrary, and often extremely short, timeframes commonly used to measure response data. With growing interest surrounding the use of behavioural endpoints for toxicity testing, these concerns must be addressed to ensure robust and meaningful science. The presentation will highlight recent research aimed at addressing some of the criticisms regarding the use of behavioural testing for aquatic toxicology. Results from a series of experiments evaluating variance between fish (*Gambusia holbrooki*) and within individual fish will be revealed, and the implications of inadequate data acquisition times for influencing toxicity outcomes will be discussed. Results demonstrate that the timeframe over which behavioural data is collected represents an extremely important consideration when designing toxicity tests, since it can dramatically influence responses and thus overall interpretations. We hypothesise that this stems from the fact that, unlike discrete endpoints (e.g., developmental stage or daily egg production), behaviour is highly dynamic and thus not adequately captured over short timeframes. The findings hold profound implications for future toxicity studies incorporating behavioural endpoints, and yield insights that may be helpful in determining which existing studies are robust and repeatable, and which are more likely to reflect anomalous outcomes. It is hopeful that the presentation will stimulate significant discussion and collaborative thought regarding the future design and application of behavioural studies in aquatic toxicology.

Predictive models in ecotoxicology: bridging the gap between scientific progress and regulatory applicability (I)

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Availability and organization of QSAR-s influences the higher understandability and acceptability of descriptive and predictive models
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Since the seminal work by Hanch and Fujita, QSAR got boost particularly in medicinal chemistry. However already before that it has been at the forefront of explaining obscure physical, chemical, biological (incl. toxicological) phenomena, and holds same position today. Many QSARs have been developed, most have found way to scientific publications that are often difficult to understand, verify and reuse. This hinders the understandability and acceptability of descriptive and predictive models. Fortunate are those very few models that have found their way to the software solutions. The current presentation gives an overview of perspective reuse of published models from physico-chemical properties to human health endpoints. It looks into repositories and integrated modelling environments available for QSAR models [1] and discusses the lifecycle of models and how models reach the intended audience from the perspective of model developers and users. The presentation discusses data formats to store QSAR model information [4] and looks more specifically into experience of QSAR DataBank approach and interactive web repository [3], http://qsardb.org/, that stores models in an open QsarDB data format [4], that is a general chemoinformatic solution for the electronic organization and archiving of QSAR information according to open standards. The repository is smart; allows visualization of data and models and has prediction functionality and estimates for applicability domain and provides prediction reporting. For QSARs with integrated descriptor calculator web service for predictions is available. Presently repository contains over 400 unique models, for about 70 endpoints and for about 20 species. Available QSAR-s utilize from simple regression models to complex classification, decision tree, neural network, random forest, support vector machine and consensus model mathematical representations. What most important such repository allows easy access, understandability and resulting acceptability of descriptive and predictive models for all user groups. **References:** 1. Tetko IV, Maran U, Tropsha A. 2017. Mol Inf 35: eraly view 2. Sild S, Piir G, Neagu D, Maran U. 2017. in Big Data in Predictive Toxicology, Eds. Neagu D, Richarz A, Royal Society of Chemistry, in press 3. Ruusmann V, Sild S, Maran U, 2015. J Cheminfor 7:32 4. Ruusmann V, Sild S, Maran U, 2014. J Cheminfor 6:25 **Acknowledgement:** Estonian Ministry of Education and Research: grant IUT34-14

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Data integration to increase quality and reliability of QSAR predictions
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REACH requires information on ready biodegradation, which is a screening test for the assessment of biodegradability and encourages the use of a weight-of-evidence approach, including predictions from QSARs (Quantitative Structure-Activity Relationships). Thus, several QSAR models have been calibrated to predict biodegradation. However, the decision-making processes in the regulatory context is often associated to limited or contradictory information. In this framework, data prediction require the use of an appropriate Intelligent Testing Strategy (ITS), which can integrate heterogeneous information collected by several methodologies, including QSAR. This can lead to the increase of reliability of the final outcome and represent a tool to obtain a greater confidence when dealing with QSAR predictions. The aim of this study was to develop a consensus approach to integrate in-silico predictions for ready biodegradability to optimize available information by the combination of estimations of several models to improve quality and reliability of QSAR predictions. Integration was carried out with eight different QSAR models. An extended set of 416 molecules, not included in the training sets of the considered models, was selected. Predictions were combined with both basic (majority voting schemes) and advanced (Dempster-Shafer's theory of evidence and Bayesian consensus) consensus approaches. Classification performances of both individual QSAR models and consensus approaches were compared Results indicated that the application of consensus methodologies show evident benefits when sources of information are jointly analysed, both in terms of classification scores and percentage of predicted chemicals. Moreover, advanced consensus approaches led to a reduction of uncertainty associated to biodegradability predictions. Results were further analysed by evaluating the relationship between chemical structure of substances and both QSAR and consensus predictions, in order to evaluate how misclassifications were related to specific structural characteristic of chemicals. Results demonstrated how the application of consensus approaches can improve the reliability of predictions obtained using individual QSAR models. Data integration and supplementary chemical characterisation can significantly increase the confidence of the final user on the QSAR outcome, thus providing additional information to augment reliability associated to QSAR predictions, and consequently their acceptance.

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CATALOGIC 301C model - validation and improvement
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The growing integration of chemistry in the modern daily life put foreword the necessity of the assessment of their fate in the environment and effect on humans. In this respect, information on the degradation potential of chemicals in the environment is required in a number of initiatives on national and international levels (such as REACH in Europe and Chemical Substances Control Law (CSCL) in Japan). Considering the number of substances included in national and reginal inventories of substances, time and cost effectiveness of the tests, the use of of non-test methods was recomended. In Europe, REACH legislation encourages the use of alternative in silico methods such as (Q)SAR models. According to the recent progress of CSCL in Japan, (Q)SAR predictions are also utilized as supporting evidence for the assessment of bioaccumulation potential of chemicals along with read across. Currently, the effective use of read across and QSARs is examined for other hazards, including especially biodegradability. CATALOGIC 301C model is a subject of regular validation and improvement with data for existing and new tested chemicals by the National Institute of Technology and Evaluation (NITE) of Japan. This study describes the results of external validation and improvement of CATALOGIC 301C model based on more than 1000 tested new chemical substances of the publication schedule under CSCL. BOD values, and for some of the tested chemicals quantities of the parent chemical and major metabolites at the end of the test, were provided by NITE Japan. Detailed biodegradation information provided by NITE allowed validation and improvement to be focused not only on presistency prediction but also on prediction of degradation products. Validation results showed that for in domain chemicals the sensitivity (i.e. predictability of non-ready degradable chemicals) and specificity (i.e. predictability of ready

degradable chemicals) were about 85% and 70 %, respectively. Significantly reduced specificity was obtained for out of domain chemicals. All documented metabolites for about 60% of 196 chemicals with observed netabolism were correctly predicted. As a result of the updating process of the CATALOGIC 301C model improvement of predictability was achieved both for observed BOD values and documented biodegradation pathways. The curent training set of the model contains aproximatly 2580 observed BOD values and documented degradation products for about 750 chemicals.

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Towards more interpretable QSARs: Lessons Learned from Aquatic Bioaccumulation Models
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Quantitative Structure-Activity Relationship (QSAR) models link a biological/environmental property of interest to a set of theoretical molecular descriptors, through chemometric/statistical techniques. Despite their high predictive capability, two aspects often hamper their acceptance by stakeholders: (1) the nature of molecular descriptors selected, and (2) the complexity of the chosen modelling technique. This work discusses some useful strategies to leverage the molecular descriptors and the model interpretation into more understandable QSARs, connected to a potential mechanistic interpretation. In particular, two modelling targets were addressed: the Bioconcentration Factor (BCF) and the Biomagnification Factor (BMF) on fish. For what concerns BCF, we first developed a QSAR scheme to predict whether a compound is (1) well predicted by K_{OW}, (2) underestimated by K_{OW}, or (3) overestimated by K_{OW}. The approach was based on two classification trees [1], whose salient features are descriptor interpretability and simplicity. The molecular descriptor interpretation allowed us to glean new mechanistic insights into the bioconcentration. The developed classification tool was then used to combine the advantages of three existing models on each predicted class, in order to maximize the accuracy of BCF prediction towards unknown data. The obtained expert system showed an increased performance than the individual models [2] (Root Mean Squared Error in Prediction on external data = 0.54), with the advantage of interpretability and connection to a potential bioconcentration mechanism. Also in the case of BMF, we struggled to find the best compromise between complexity and prediction accuracy, by choosing simple modelling techniques and striving to interpret the selected descriptors. The developed model led to accurate BMF predictions and the descriptor interpretation allowed investigating and rationalizing the structural features responsible for the biomagnification of organic chemicals within the food web. **References** [1] Grisoni F, Consonni V, Vighi M, Villa S, Todeschini R. 2016. Investigating the mechanisms of bioconcentration through QSAR classification trees. Environ Int 88:198–205. [2] Grisoni F, Consonni V, Vighi M, Villa S, Todeschini R. 2016. Expert QSAR system for predicting the bioconcentration factor under the REACH regulation. Environ Res 148:507–512.

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Can in silico approaches increase confidence in environmental risk assessment methodology? Some case studies
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The current environmental risk assessment paradigm is based on theories, approximations and extrapolations. For many endpoints uncertainty factors are added in order to reduce the probability of underestimation of risk. The pertinence of these factors has been the subject of controversy for many years: Does a toxicity value determined in a short term study reflect the toxicity across species likely to be in contact with the substance (incurring a factor of 10 in risk assessment Is an Acute to chronic ratio a reasonable concept for ecotoxicological endpoint extrapolations (also often associated with a factor of 10)? Can reactive chemical toxicity be determined in a consistent way? How can we use read-across for substances that change their mode of action within a category? Can we still get a grip on concepts that cannot be resolved analytically? How valid are the data used in the risk assessment? This presentation focuses on these points, supported by *in silico* methodologies which introduce the concept of High Accuracy QSARs (HA-QSARs) linked with Mechanisms of Action (MechoAs), and provides some case studies which demonstrate how these methods can be used to go beyond simply predicting toxicity values to actually help resolve and increase confidence in the questions posed above on which much of the current risk assessment paradigm is founded: Using quantic descriptors to categorise substances into specific (MechoAs), and then linking these to ecotoxicity values, downstream of species dependent Adverse Outcome Pathways (AOPs) Acute to chronic ratios (are they MoA/MechoA dependent?); A simple proposal to account for and quantify reactive chemical toxicity in aquatic ecotoxicity Separation of specific structural groups (which would normally be identified as a category) into different MechoAs with different toxicities Cases where *in silico* approaches can be used to conclude on the presence of phases that cannot be measured analytically "*In silico* Cascade approaches" for data validation Conclusion: *in silico* methods used in the current risk assessment paradigm are primarily used to predict experimental results and are received by industry and regulators alike with gradually increasing warmth. However, their true potential to resolve sticking points in risk assessment methodology has yet to be recognised.

Highly Hydrophobic Chemicals: Reliable Investigations on Environmental Fate and Effects

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Analysis and ecotoxicological investigations of poorly soluble cosmetic compounds
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Highly hydrophobic chemicals (e.g., log $K_{ow} > 5.5$ and aqueous solubilities in the low μg/L range) are in widespread use, ranging from industrial applications to consumer products like cosmetics (personal care products). Due to high production volumes and their typical 'rinse off' application, substantial amounts of these chemicals end up in the waste-water and may subsequently enter aquatic ecosystems. This causes the need for reliable assessments of their environmental fate and toxicity to organisms as requested by the European REACH Regulation. Standard ecotoxicity tests are often not suitable to test these chemicals, since their high hydrophobicity and lipophilicity results in extensive adsorption to surfaces like test vessels and organisms. The lack of consistent and reliable results, due to the difficulties in maintaining constant test concentrations, may lead to improper assessment of possible environmental risks. The aim of this project (ECOSM) was to develop ecotoxicological methods and suitable analytics for testing highly hydrophobic substances, followed by a critical evaluation of the "poorly solubles approach" proposed by Tolls et al. (2009). The hypothesis of the approach is that substances with a narcotic mode of action and a water solubility below a toxicity threshold ($ETNC_{aqua}$) do not exhibit any acute or chronic aquatic toxicity (cf., hydrophobicity cut-off). Dodecylbenzene (DDB) has been selected as a first model compound. It is liquid under standard conditions and highly hydrophobic (log K_{ow} 8.65). Passive dosing was selected as a delivery tool for the test substance in aquatic ecotoxicity test systems and provides controlled release of the test substance while compensating for losses. The maximum solubility of DDB in pure water, algal medium, and Daphnia medium was determined by different techniques and compared to several estimation tools. The results confirmed a solubility of DDB in the lower μg/L range (6±2 to 24±15 μg/L) in all tested media. Beside the adaptation of existing passive dosing strategies, a new passive dosing format was developed and applied for the testing of highly hydrophobic compounds exactly at the saturation limit. DDB was found to exert moderate toxicity in algal growth inhibition assays, which was higher than expected compared to the "poorly solubles approach" and a reported hydrophobicity cut-off in toxicity.

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Should regulatory short-term aquatic toxicity tests be waived for highly hydrophobic chemicals with slow bioconcentration kinetics?
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Chemical regulations worldwide request a base set of aquatic ecotoxicity data for registration even for highly hydrophobic chemicals. However, many hydrophobic organic chemicals could be classified as baseline toxicants and their internal concentration is not likely to exceed the critical body burden during the test periods of conventional ecotoxicity tests because of slow bioconcentration kinetics even if they might be intrinsically toxic. In this study, we predicted the changes in the internal concentrations of highly hydrophobic chemicals during standard acute ecotoxicity tests at three trophic levels: algae, invertebrate, and fish. Model suggests that chemicals with an octanol−water partition coefficient (K_{ow}) greater than 10^6 are not expected to reach sufficiently high internal concentrations for exerting effects in short-term tests with fish and invertebrates. Validity of the prediction was also confirmed by compiled data of the OECD's screening information data set (SIDS) (n = 746), apart from a few exceptions concerning mainly organometallic substances and those with inconsistency between water solubility and K_{ow}. Considering uncertainites of the model, we propose a revision of data requirements for highly hydrophobic chemicals with log Kow > 7.4: Short-term toxicity tests can be limited to algae that generally have the highest uptake rate constants, whereas the primary focus of the assessment should be on persistence, bioaccumulation, and long-term effects.

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PCB and PCDD partition constants in mixed dissolved phases and the impact of the individual mixture constituents

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The impact of mixtures of dissolved phases such as surfactants and humic acids (HA) on highly hydrophobic contaminants (hHOCs, log $K_{OW} > 5.5$), many of which can be highly toxic e.g. dibenzo-*p*-dioxins (PCDDs), is unknown. However, it is recognized that due to partitioning processes dissolved organic phases can alter the aqueous solubility of hHOCs and potentially lead to groundwater contamination and offside transport of contamiants. Therefore, methodologies to quantify the mixture impact on the partitioning behaviour of hHOCs is important. Mixture behaviour of a binary anionic (C_{12}-2-LAS)-nonionic ($C_{12}EO_4$) surfactant mixtures at two mass ratios (33:67 or 83:17 $C_{12}EO_4$:C_{12}-2-LAS) as well as in mixtures with HA was assessed via polymer-water sorption isotherm, where the critical micelle concentration (CMC) is denoted by the inflection point of the sorption isotherm. Tensiometer measurements were carried out for validation purposes. A previously reported passive dosing method was applied to determine PCDD and PCB (log K_{OW} 5.8-8.3) partition constants in the different mixture solutions at total surfactant concentrations above the CMC. The CMCs of both co-surfactants in the mixtures were lower than their CMC in single surfactant solution and were dependent on the mixture ratio. The CMCs of the co-surfactants in either the binary or the ternary mixture with HA increased with increasing HA concentration. PCDD and PCB partition constants to the $C_{12}EO_4$:C_{12}-2-LAS mixtures were quantified (K_{MImix}) and found to increase with increasing hHOC hydrophobicity as reported in single surfactant solutions. Furthermore, partition constants to micelles (K_{MImix}) for hHOCs in the surfactant mixtures increased with increasing $C_{12}EO_4$ fraction in the mixture, but, stayed below the values for single $C_{12}EO_4$. A dependency on HA concentration for hHOC partition constants in $C_{12}EO_4$-HA mixtures ($K_{MI\text{-}DOC}$) was observed, where $K_{MI\text{-}DOC}$ decreased with increasing HA. In C_{12}-2-LAS-HA mixtures as well as in the ternary mixture log $K_{MI\text{-}DOC}$ and log $K_{MImix\text{-}DOC}$ were similar for the different HA concentrations as well as in comparison to reported partition constants to single surfactants or HA. Partition constants of hHOCs to the model mixtures were quantified and the mixture effect of individual dissolved phase constituents was investigated which may be promising for a wide applicability of the techniques in fields such as toxicity testing or bioavailability determination of hHOCs in the environment.

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Indirect measurements of organic carbon/water partitioning of volatile methyl siloxanes
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Volatile methyl siloxanes (VMS) are highly hydrophobic substances that include high production volume chemicals used in personal care products and in the production of silicone polymers. Understanding their fate and persistence in aquatic systems that receive direct input via discharges from sewage treatment plants requires knowledge of their equilibrium partition ratios between organic carbon and water. The key limitation to measuring organic carbon/water partition ratios is that their extremely low solubility makes analysis of the substances in water practically impossible. Here we describe an "indirect" method to determine organic carbon/water partition ratios of organic chemicals that does not rely on determining the concentration of the substances in water. We have applied the method to measure partition ratios of VMS and polychlorinated biphenyls (PCBs) between water and both particulate organic carbon (K_{OC}) and dissolved organic carbon (K_{DOC}), to measure salting out of VMS and PCBs from water to organic carbon, and to determine the temperature dependence of organic carbon/water partition ratios for VMS and PCBs. Organic carbon/water (K_{OC}) and dissolved organic carbon/water (K_{DOC}) partition ratios measured using our method for PCBs 28, 52 and 153 are in good agreement with previous measurements using other techniques, which serves as a validation of our method. Our measurements for the cyclic (D4, D5 and D6) and linear (L4 and L5) VMS are in some cases the first empirical data for these substances. Where previous measurements were available, our new measured partition ratios for the VMS are usually approximately an order of magnitude higher. Enthalpies of phase change between water and organic carbon (DH_{OC}) determined for VMS from measurements of K_{OC} at temperatures between 5 and 25 ºC indicate that VMS have a higher affinity for organic carbon at lower temperatures. Measured of the enthalpies of phase change between water and octanol (DH_{OW}) show the opposite trend, ie, lower affinity for octanol at lower temperatures. The temperature dependence of K_{OC} is thus qualitatively different than that of K_{OW} for the VMS in that the enthalpies have opposite signs. Potential future applications to other hydrophobic substances, including mixtures that are classified as UVCBs (unknown or variable composition, complex reaction products or biological materials) under the European Chemicals regulation REACH will be discussed.

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Simulation of bioremediation options by microbial degradation of aged (PAH) contaminations in soils
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At many sites contaminated with hydrophobic organic chemicals such as PAH the pollutants partly sequester with time remaining unavailable for biodegradation. Residual tar oils containing PAH are often dumped combined with small grain size waste coal and coke materials from processing at former gas work sites. In order to find suitable solutions for improving the biodegradation potential at the respective sites, predictions can be provided for the consequences of altered conditions for microbial degradation. For optimising remediation strategies and for interpreting failures, a combined model was applied for simultaneously considering dissolution from an organic chemical phase (NAPL), ad/desorption, sequestration (aging), microbial metabolism and growth, and the formation of non-extractable residues. The model was used for the simulation of bioremediation options for clean-up of PAH-contaminated soils. The objectives were to understand the behaviour of PAH in contaminated environments and to give recommendations for bioremediation measures. We analysed the turnover of PAH by combining ad/desorption models for organic compounds with models for the growth and degradation kinetics of microbes. We modelled several scenarios and interpreted the observed effects, such as increasing distribution coefficient (Kd) and persistence of the PAH with time, decreasing degradation rates with concentration, and effects of amendments on sorption and degradation. Based on the kinetics of the processes and the fluxes in the system, we can provide a robust mathematical definition of the terms "bioavailability" and "bioaccessibility". Finally, the model was applied to evaluate the most effective remediation strategy for PAH contaminated soils and sediments.
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Advances in Soil Ecotoxicological Risk Assessment of Chemical Stressors (I)

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Springtails: the canaries for soil pollution
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The Dutch governement has determined a list of sites that potentially have ecological risks due to soil pollution. These risks have been determined by a computer model which hardly is site specific. For site specific risk assessment the Triad can be used. ALthough this method is suited to assess risks at a site, it does not explain the cause of the effect or risk. Therefor, within the BE-Basic program, VU Amsterdam has developed a test that is able not only to assess an ecological effect but also the cause of the effect: the invertebrate Soil Quality test with springtails. Springtails are sensitive to all kinds of pollution. The presence of pollution leads to a stress response that is pollution specific. This stress response is measured by PCR on specific proteins that code for specific enzymes. This enables us to distinguish effects caused by for instance PAH by stress cuased by metals or extreme pH, even when exposed simultaneously to all. The amount of proteins found can be linked to effects on reproduction. In this presentation we will explain the mehanism of the test and we will give three short examples of sites at which this test was used to make a decision about the site. The first site is situated in the dunes. The site has been elevated and leveled out with domestic waste which has caused a severe soil pollution with heavy metals. However, based on a so-called social consideration (which also includes non soil parameters) ecological risks were not expected, even though the model had indicated severe risks. The test was used to objectify this consideration. The second site is a former flow field from the textile industry which is polluted with chromium. A Triad approach has been performed. Risks were found but could not be linked to the pollution. The third example is a former landfill at which also a Triad apporach has been performed. High concentrations of metals and PAH were present. The iSQ test could link risks to soil organisms to PAH exposure. By means of the examples we will show that the iSQ test can be used to assess ecological risks and the cause of that risk. The test is applicable to all kinds of situations: as part of the Triad approach, objectifying of social consideration, additional support for (previously determined) remediation values, prioritizing remediation of sites based on actual ecological risks. Remediation of sites can thus be limited to those sites where risks are actually present.

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Specifying the applicability boundaries of the Equilibrium Partitioning Method (EPM) within a regulatory context: A way to go forward
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According to REACH, the equilibrium partitioning method (EPM) may be applied in some cases to assess the hazard to soil and sediment organisms based on results from aquatic toxicity assessment. While the EPM is supposed to be used primarily as a screening tool for establishing the need of further testing on soil and sediment organisms, in reality it is often used for decision-making without being supported by test results. Based on the information extracted from the REACH database up to this point, only about 13% of registration dossiers have $PNEC_{soil}$ derived from experimental terrestrial toxicity data - while over 35% have PNECs estimated by using the EPM. The extent to which EPM is used for $PNEC_{soil}$ calculation underlines the importance of having a further insight on its applicability within the regulatory context. In October 2016 the European Chemicals Agency (ECHA) launched a regulatory science research project on the EPM applicability boundaries. The project aims at collecting available data from the REACH registration database, dossiers evaluated under Biocidal Products Regulation (BPR), and additional information from project partners (ECETOC, ExxonMobil and Environment and Climate Change Canada). The objective is to identify when the screening approach is adequate, what are its limitations, and when further testing is needed to assess the toxicity in soil and sediment organisms. In addition, the project aims to address the potential for alternative screening tools within REACH and Biocides processes. The first phase of the project (October 2016 – March 2017) covers the screening of available sources and a preliminary analysis of collected information (e.g., psysicochemical properties, chemical structure, aquatic / sediment / terrestrial toxicity). As of November 2016, the dataset covers only REACH database, taking into account registration dossiers submitted to ECHA from June 2008 to August 2015. The dataset will be expanded with latest REACH registrations and additional sources by March 2017. Prior to compiling the integrated dataset, data filtering will be conducted taking into account the quality of available data. The aim is to keep reliable high-quality sources in order to minimize the effect of experimental artefacts and reach robust conclusions on the applicability domain of EPM. This presentation outlines the project's scope and methodology, and presents the preliminary findings based on up-to-date screening of the available information.

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Considering the what, why, how and where in Soil In-Field Protection Goal derivation

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In its recent draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products (PPPs) for in-soil organisms, EFSA proposes options for protection goals (PGs) based on the Ecosystem Services (ES) concept. The Commission needs to select PGs in consultation with risk managers, considering a cost-benefit evaluation, socioeconomic and political criteria acknowledging consequences for human well-being (health and economic benefits) as well as any environmental impact. The PGs based on environmental and ecological criteria initially proposed to risk managers need to be viable in the context of agriculture and not simply prohibiting factors. While in the Scientific Opinion it is acknowledged by the Panel that the in-field crop protection might be rated higher than biodiversity in terms of provisioning services, a PG related to in-field biodiversity is still set out. A broad range of substances are expected to fail the in-crop risk assessment due to initial effects seen at field rates. This PG will impede food production/crop protection as a service,as field rates will need to be reduced to below the required efficacy levels in order to meet it. It is also demonstrated that impacts on crop protection are also expected due to proposed conservative run-off estimations, which consequently may require significant in-crop buffers for reducing off-field exposure. The assessment of biodiversity as a PG is challenging, due to the difficulty in deriving appropriate 'normal operating ranges' for in-field areas in agricultural landscapes (vaguely defined in the Scientific Opinion and arbitrarily chosen reference systems for its derivation, i.e. extensively managed organic farmed fields with low input of PPPs). Available realistic field data demonstrate that natural variability of in-field organisms, such as Collembola, are very high in abundance and community structure, and independent of spatial scale. It is proposed that the in-field PGs should be based on soil functional, rather than structural, endpoints. The focus for an in-field risk assessment should be set on protecting ESs for maintaining the basis of food production while protecting the soil functions. In a presented field study, it is demonstrated that not all in-soil taxonomic groups contribute equally to soil functionality. Soil functionality of the respective taxonomic groups should be considered and the in-field risk assessment should focus on the relevant groups.

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Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms: specific protection goals and research needs

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The Panel on Plant Protection Products and their Residues (PPR) has been tasked by EFSA to revise the SANCO guidance Document on Terrestrial Ecotoxicology which is currently used for conducting the risk assessment for terrestrial non-target organisms, except for birds and mammals and non-target arthropods. For each group of organisms, the PPR Panel first summarises the science behind the respective risk assessment in a scientific opinion and, in a second step, EFSA will develop practical guidance on how to perform the risk assessment. The main scope of the revision is to take into consideration: (i) the entry into force of the Regulation 1107/2009 replacing the Directive 91/414/EEC; (ii) the need for developing specific protection goals and (iii) the inclusion of new scientific elements in the environmental risk assessment of in-soil organisms. The Opinion only covers chemical plant protection products (PPPs), while microbial PPPs are not specifically addressed in the opinion. The working group of the PPR Panel reviewed the current environmental risk assessment, identified key drivers that sustain important soil ecosystem services in agricultural landscapes and developed proposals for specific protection goal (SPG) options for in-field and off-field areas, according to the procedure developed in the opinion of the PPR Panel of 2010 Based on the overarching ecosystem services concept, which was boosted in the so-called Millennium Ecosystem Assessment, those ecosystem services that could potentially be directly or indirectly (e.g. via trophic interactions) affected by the normal agricultural use of plant protection products were first identified, followed by the groups of in-soil organisms that are key drivers or service providing units (SPUs) for those ecosystem services. Specific protection goal (SPG) options have to be proposed for each combination of a key driver and ecosystem service. During the development of the opinion, several research needs covering different aspects in the context of pesticides risk assessment for in-soil organisms have been identified. Some of those needs, such as standardisation of toxicity tests with additional species than the standard ones, should be prioritised before the development of the guidance document. A public consultation on the draft opinion was launched in May, 2016 aiming at involving all the interested stakeholders.

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Framework of tiered risk assessment approach of pesticides for soil organisms in China

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The aim of the risk assessment for soil organisms is to prevent negative acute or long-term effects on soil functions and functionally most important species. The risk assessment approach established in China considers ecologically relevant groups of soil organisms, eartheworm and soil microorganisms, which are involved in a range of soil functions providing essential ecosystem services, e.g. organic matter breakdown and mineralization, water regulation in soil. The tiered approach is a valuable tool to quickly identify those pesticides which do not pose acute or chronic risk (in a certain area of ecotoxicology) on soil organisms – even under worst case assumptions, and to identify those that need more attention and further evaluations. All risk assessments presented are based on Risk Quotients (RQ), calculated by dividing the Predicted Environmental Concentration (PEC) by the Predicted no Effect Concentration (PNEC). This calculation takes into account, that beside the toxicity of a pesticide, the amount of this pesticide in the environment plays a major role when assessing a risk. If RQ > 1, the risk is unacceptable and higher tier risk assessment should be conducted. Exposure analyses employ tiered assessment approach. Tier 1 exposure analysis employs simple model (PECsoil_SFO_China from NIES) to predict exposure to soil organisms. A higher tier exposure analysis can be applied by refining environmental exposure parameters or using semi-field trial test. Currently, the models PRAESS and China-PEARL, which developed by NIES and ICAMA in China, are applicable to predict the exposure concentration at specific depth of soil layer and at specific scenarios in China. Proposed test systems for effect assessment include acute toxicity test or reproduction of earthworm, reproduction test, nitrogen transformation test, litterbag test and earthworm field test. The PNEC can be calculated using the endpoint obtained from ecotoxicological studies and corresponding uncertainty factors (UF). Tier 1 risk assessment mainly focuses on the earthworm acute or chronic (in case pesticide DT_{50}>180 d or RQ_{acute}>1) assessment and N - transformation assessment. High tier risk assessment mainly

foucses on the litterbag test assessment and earthworm field assessment. We have used this tiered risk assessment approach to assess the risk of more than 40 common used pesticides in China. **Keywords:** tiered, risk assessment, pesticide, soil organism

Challenges and best practice in monitoring of micro- and nano-plastic abundance and environmental distribution (I)

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Current methodologies for the isolation of microplastic pollution cannot account for all domestic microplastics

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The wastewater treatment process has long been recognised as a potentially major source for two forms of microplastic pollution: cosmetic microplastics and synthetic fibres, herefafter ‘domestic microplastics’. A small number of publications have documented the emission of microplastics from wastewater treatment plants (WWTPs) across Europe and North America, and, whilst these report that the majority of microplastics are retained by the wastewater treatment proces, the cumulative emission of microplastics into the receiving waters of WWTPs represents a potentially major source of microplastic pollution. Established methodologies for the quantification of domestic microplastics in WWTP effluent and their receiving waters fail to account for the contamination of samples by airborne synthetic fibres and overlook the diversity of sizes in which domestic microplastics are known to occur. Here an alternative methodology for the minimisation of of synthetic fibre contamination in the processing of aquatic samples is discussed and the influence of the morphology of domestic microplastic particles is considered in the context of their isolation from environmental samples.

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The use of reference materials in microplastic research: general aspects

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Discussions in science, politics and public media about microplastic in the environment and its unclear effects for animals and humans are common these days. Although microplastic findings in water, soil and even air are well known, no general estimations about the quality or quantity of microplastic particles in environment exit. Research is necessary to develop reliable and traceable, harmonized protocols for sampling, sample preparation and fast, but accurate analysis for qualitative and quantitative measurement of microplastic in various environmental matrices. For development of such reliable and traceable methods, the use of microplastic reference materials is necessary to assess various concepts. These microplastic reference particles should be an appropriate reflection of the microplastic particles found in environment. Due to the high use in industrial products six polymers seem to be relevant in environment as microplastic particles: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET), polyamide (PA) and polyvinylchloride (PVC). The raw source of these thermoplastic materials are pellets or granulate with dimensions of 1 to 5 mm in diameter. Most common they own a regular cylindrical shape or are prolate sphericals. These can be bought cost-efficient by various polymer producers. But discussions in microplastic research topic lead to particle sizes down to 1 μm. Furthermore, various unregular shapes and geometries of the particles have to be considered as well as an oxidized surface. In the present work we engage the acquisition and production of realistic reference material. Different aspects should demonstrate the complex and difficult task of generating approriate, cost-efficient and well-defined reference materials. The particle size distribution by using different particle sizing methods as well as individual particle shapes are documented. Different material properties were addressed. PE, PS and PA were chosen as typical and relevant microplastic with opposite individual material characteristics in density and glass transition temperature. The easiest possibility for acquisition of small microplastic particles is the purchase of polymer emulsion particles (20 to 150 μm). An alternative possibility to produce small microplastic particles is milling under liquid nitrogen conditions in cryo mills. Particles purchased or self-made differ strongly in shape and size depending on production method.

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The influence of different sampling and extraction procedures when quantifying microplastics in beach sand

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Human plastic consumption has increased at a dramatic rate over the last decades, and a significant proportion of this plastic enters the environment. One subgroup of emerging concern are small pieces of plastic, termed microplastics. Microplastics are ubiquitous in the environment, are frequently ingested by organisms, and may potentially cause harm. A range of studies have found significant levels of microplastics in beach sand. However, there is a considerable amount of methodological variability among these studies. Methodological variation currently limits comparisons as there is no standard procedure for sampling or extraction of microplastics. We identify key sampling and extraction procedures across the literature through a detailed review. We find that sampling depth, sampling location, number of repeat extractions, and settling times are the critical parameters of variation. Next, using a case-study we determine whether and to what extent these differences impact study outcomes. We found that sampling procedures have limited impact on the study outcomes. However, when extracting microplastics the settling time and number of repeat extractions need to be standardized for comparison across studies. By investigating the common practices identified in the literature with the case-study, we provide a standard operating procedure for sampling and extracting microplastics from beach sand.

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Analysis of microplastics by LC-QExactive Orbitrap in coastal areas of Catalonia

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The enormous quantity of plastics in in coastal waters, estuaries and oceans waters is one of the serious problems of the current epoch. Consequently, under environmental conditions, plastic items are able to generate smaller fragments arriving to nano- and microscopic sizes (known as nanoplastics (NPLs) and microplastics (MPLs), respectively)[1]. Accordingly to the type of plastic, the density of these particles can change and this may influence the bioavailability in the water column; thus, the presence of these contaminants in sediments as well as the polymers ingested by marine organism may vary[2]. In addition, rivers could transport MPLs to the downstream of the lakes and coastal environment, where they can interact with biota[3], arriving in human diet. Simultaneously, MPLs can transport toxic plastic additives or collect organic contaminants and pathogens from the surrounding sea water[4]. The main objectives of this work were: I) the optimization of the method by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) to determine the presence of the two most frequently used plastics (polyethylene and polystyrene); II) to obtain information about the occurrence of some plasticizer and additives by the so-called non-target analysis; and III) to apply the methodology in order to assess the presence of selected compounds in water, sediment and biota samples from Mediterranean estuarine coastal area (Ebro Delta from Catalonia, Spain). Due to the nature of MPLs, size exclusion chromatography coupled to APPI-HRMS-QExactive can be used for characterization of monomer types but also a quantitative approximation due to the mass spectrometer employed. This technology allows the comparison of MPLs concentrations in environmental matrices (waters, sediments and biota).

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Separation and identification of tyre debris in environmental samples

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Tyre debris particles belong to the group of microplastics and might account for a large portion of microplastic pollution. While the input of tyre debris into the environment is assumed to be high, few studies have actually measured tyre debris particles in environmental samples up to now. The aim of the present study is to develop and apply a two-step analytical protocol which is based on separation of tyre debris from the environmental matrix, followed by identification and quantification via elemental analysis. Tyre debris is expected to be identifiable and quantifiable because of its characteristic elemental composition (e.g. S and Zn content), allowing the distinction from other synthetic organic polymer particles. In the present study, a rarely used separation method consisting of a pre-concentration step and subsequent density separation is being evaluated. Separated particles are further subjected to elemental analysis. Pre-concentration is accomplished by flotation in a fluidized bed reactor. The pre-concentrated sample then undergoes a digestion treatment in order to oxidize biogenic matter. Isolated particles are further separated by density separation and are eventually filtered and dissolved with the means of microwave assisted acid digestion. Dissolved elements are analysed via inductively coupled plasma atomic emission spectroscopy (ICP-AES). First tests indicate a recovery of 94.7% for the pre-concentration and density separation with sodium polytungstate. Recoveries for the digestion step are within 21%-129% but are expected to improve with changes in the sample treatment. Recovery experiments for the whole sample preparation will be conducted with spiked artificial and environmental samples. Currently, analysis of a set of tyres is conducted in order to validate and extend an existing data set of elemental compositions. Tyres will also be analysed for the contents of C, H, and N in order to gain more information on elemental compositions. The method will then be applied to environmental samples, especially from urban areas. The presented sample preparation procedure has potential to become a reliable method that enables the isolation of tyre debris particles from environmental samples. In the future, analysis of single particles by coupling a laser ablation system to a mass spectrometer is planned. *Acknowledgement* - The authors thank the BMBF for funding the MiWa project (reference number 02WRS1378H) as well as our project partners.

Toxicology and Ecotoxicology, human and ecological risk assessment of engineered nanomaterials: needs, goals and tools/methods for safer-by-design strategies

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Grouping of nanomaterials regarding their behaviour and fate in the environment - hypotheses and experimental results
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Engineered nanomaterials are used in different products with the consequence that they can be released into the environment during their life cycle. Given the large varieties of ENM, the effort for the individual investigation and assessment would be enormous. Therefore a grouping of ENM and read across between different materials is one major target for future risk assessment. Here we will present usable and practicable approaches for a grouping of ENM regarding their fate in the environment elaborated within nanoGRAVUR. In a frist step parameter for a grouping of ENM in air, water and sediment/soil regarding their transformation or mobility were identifed based on a literature review. Depending on the environmental compartment solubility, zetapotential, surface chemistry, (primary)particle size, morphology, chemical composition, polarity and density were identified as important parameters for a possible grouping and read across of ENM regarding their fate and behaviour. Based on the conducted review it seems that the surface properties are of major relevance for a grouping of ENM. If this hypothesis holds true under simulated environmental conditions will be tested in nanoGRAVUR in the next step. Here different experiments for verification or falsification of the identified parameters will be conducted with so far ungrouped ENM. First results will be presented at the conference. In air the transformation of ENM will tested in a wind tunnel according to DIN 71460. Experiments will be conducted at different humidity, UV irradiation and temperature. The transformation in water and soil like the change of the surface chemistry of the ENM will be tested within a selected range of ionic strength, pH and NOM concentration in the media. The agglomeration / adsorption and sedimentation behaviour will be studied as grouping paramater for the mobility in water and soil. Based on the results the developed approach for a grouping will be adapted, if necessary. *Acknowledgement* - The results are generated in the framework of the project nanoGRAVUR which is funded by the German Federal Ministery for Education and Research (BMBF) under grant no.: 03XP0002

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Non-additive mixture effect decrease wastewater toxicity due to micropollutants adsorption on inorganic nanoparticles
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One of the most serious environmental problems is the continuous input of hazardous pollutants into surface water through wastewater. Even at very low concentration, micropollutants are potentially toxic to freshwater organisms[1]. Metal oxide nanoparticles are increasingly used in a wide spectrum of products and applications, which have led to an increased release of nanoparticles into the aquatic environment. Specifically, due to their high specific area and reactivity, nanoparticles may adsorb compounds on their surface and act as carriers for waterborne micropollutants, modifying their biodegradability, mobility and toxicity[2]. We studied the adsorption of micropollutants from a real biologically-treated wastewater effluent on nano-oxide nanoparticles of silica, amine-modified silica, titanium dioxide, and magnetite. The toxicity of nanoparticles and wastewater was assessed using the bioluminescence inhibition of the bioluminescent cyanobacterium *Anabaena* sp.7120 CPB4337. The interactions of wastewater micropollutants and nanoparticles were assessed using the combination index (CI)-isobologram method. The total amount of compounds adsorbed on nanoparticles was 13.4 μg/g, 4.83 μg/g, 10.8 μg/g, and 7.13 μg/g for SiO_2, SiO_2-NH_2, TiO_2, and Fe_3O_4 respectively. The mixtures of wastewater with SiO_2, SiO_2-NH_2, and TiO_2 nanoparticles displayed antagonism for the lower affected fractions, which corresponded to the lower exposure concentrations. For the higher range of affected fractions and for Fe_3O_4 nanoparticles over the whole range tested, the mixtures were additive displaying a slight tendency towards synergism. The reduced toxicant bioavailability due to the adsorption of wastewater micropollutants on the surface of nanoparticles proved relevant at environmental concentrations and can take place with naturally occurring nanoparticles.
References [1] Rodea-Palomares I, Gonzalez-Pleiter M, Gonzalo S, Rosal, R, Leganes F, Sabater S, Casellas M, Muñoz-Carpena R, Fernández-Piñas F. 2016. Hidden drivers of low-dose pharmaceutical pollutant mixtures revealed by the novel GSA-QHTS screening method. Science Advances 2:9. [2] Fries E, Crouzet C, Michel C, Togola A. 2016. Interactions of ciprofloxacin (CIP), titanium dioxide (TiO_2) nanoparticles and natural organic matter (NOM) in aqueous suspensions. Science of The Total Environment563–564:971-976. *Acknowledgement* – This research was supported by CTM2013-45775-C2-1-R and CTM2013-45775-C2-2-R grants from MINECO.

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Decision support system for nanotechnology risk assessment and management
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In order to help industry and regulators make informed decisions about managing the risks from nanotechnologies, the Sustainable Nanotechnologies (SUN) EU FP7 project developed a Decision Support System (SUNDS) and implemented it as a web application. This software performs risk assessment and socioeconomic analysis to help its users assess the risk-benefit ratio of nano-enabled products. SUNDS consists of two tiers that differ in the complexity/specificity of the performed assessments and the stakeholders they target (e.g. SMEs, insurance companies, industry, regulators) (1). Moreover, the TÜV SÜD CENARIOS® risk management standard was incorporated as a stand-alone module in the system (2). The first tier is based on the NanoScan model, which was developed in the EU FP7 LICARA project specifically for SMEs (3). The second assessment tier is based on an adaptation of the authorisation process required by the EU REACH regulation and is aimed at supporting industrial and regulatory decisions. It consists of Risk control (RC) and Socio-economic Assessment (SEA) modules. The RC approach is based on the REACH Chemical Safety Assessment, while the SEA considers the triple bottom line approach to estimating environmental, economic, and societal benefits. The RC module incorporates nano-specific occupational and consumer inhalation and ingestion as well as environmental exposure and dose-response models for estimation of human and ecological risks. The web application software has been mainly programmed in the ECMAScript 2016 programming language by the use of the Meteor framework. The underlying architecture is composed by a Node.js server equipped with a MongoDb No-Sql database. Results are presented in a user friendly graphical user interface which uses dynamic charts provided by the Highcharts framework. SUNDS has been tested in two case studies, i.e. nanoscale Copper Oxide -based biocidal paint and plastic car bumpers coloured with Ferrari-red organic nanoscale pigment.

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Risk analysis and technology assessment in support of technology development; putting RRI in practice in a case study for nanotechnology
A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health; H. van Lente, Maastricht University; H. Bouwmeester, WUR; A. Sips, National Institute for Public Health and the Environment RIVM
Governments invest in ‘key enabling technologies’ such as nanotechnology, to solve societal challenges and boost the economy. At the same time governmental agencies demand for risk reduction to prohibit any often unknown adverse effects, and industrial parties demand for smart approaches to reduce uncertainties. Responsible Research and Innovation (RRI) is therefore a central theme in policy-making. Risk analysis and technology assessment, together referred to as RATA, can provide a basis to assess human, environmental and societal risks of new technological developments during the various stages of technological development. This can help both governmental authorities and innovative industry to move forward in a sustainable manner. Here we describe our experiences to bring RATA in practice within a large Dutch nanotechnology consortium, as an example of putting RRI in practice as integrated part of a research program.

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Poster spotlight: WE185, WE186, WE187, WE190

The Role of Metals in Circular Economies: A Life Cycle Perspective

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Development of a new industry lead Life Cycle Inventory for cobalt and perspectives on the metal LCA harmonisation effort

J.M. Fry, ERM; C. Pettit, Cobalt Development Institute / Regulatory & Sustainability; S. Aumonier, ERM

There is a continued strong growth in demand for cobalt in rechargeable batteries. In response to this increased interest, the Cobalt Development Institute (CDI) is taking a Life Cycle Approach, and applying the appropriate tools, to illustrate the role of cobalt in the circular economy. Available life cycle inventories (LCI) for cobalt had limited representativeness and varied considerably in the life-cycle results obtained, providing a limited and confusing picture of the environmental impacts of cobalt production. In response, the CDI initiated the cobalt sector's life cycle assessment (LCA) programme and has completed its first industry-lead cradle-to-gate LCI for refined cobalt in 2016. The ISO 14040 compliant assessment incorporated the guidance provided in the multi-metal LCA harmonisation document, the development of which had included the participation of the CDI. The cobalt environmental profile is being developed to illustrate the positive environmental aspects achieved through recovery/recycling/reuse for the circular economy, and the socio-economic benefits and value chains for many uses of cobalt substances. Cobalt has a role within many sectors, and the new LCI for refined cobalt is the CDI's firs step towards improved characterisation of the overall circular model (system) for cobalt. This session describes some of the conclusions drawn from this LCA and observations in applying the metal LCA harmonisation guidance.

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Life Cycle Assessment of Primary Aluminium Production

L. Wu, P. Nunez, International Aluminium Institute

The International Aluminium Institute (IAI) collects, analyses and publishes life cycle inventory (LCI) data for use by life cycle assessment (LCA) practitioners through the IAI website and professional databases every five years. The Institute has conducted a partial life cycle assessment from cradle-to-gate for 2010 data and 2015 data to evaluate the significance of potential environmental impacts, based on the LCI results, against a defined set of impact categories which can be tracked over time. Downstream manufacturing, use phase and end-of-life were excluded from the scope of this study although the LCI data from this study can be used for full LCAs of aluminium containing products. Impact modelling using the 2010 data highlighted that energy has a significant influence on the overall environmental impact in every imapct category. The 2015 LCI is an update to the 2010 LCI with the addition of regionalised datasets representing regional averages of process inputs and outputs. The IAI's LCI datasets, available in Ecoinvent and Gabi, can be considered the most accurate and up-to-date of any available LCI on primary aluminium production and should be used by LCA practitioners in LCAs of aluminium containing products where specific data is not available.

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Cradle-to-gate inventories of primary metals: update and expansion of the ecoinvent database

D.A. Turner, A. Haarman, R. Hischier, EMPA / Technology and Society Lab

Metals are ubiquitous in society and there is an ever-increasing reliance on critical or geopolitically scarce metals in modern technologies and materials. The extraction of metals from the Earth's crust and their subsequent processing can also lead to substantial environmental impacts. Metals are produced in complex, interconnected systems with the production of many critical and geochemically scarce 'companion' metals occurring as a by-product of the production of one or more 'host' metals. For LCA practitioners to better understand the role of metals production on the environmental impacts of their reference systems, it is important that high quality, up-to-date life cycle inventory (LCI) data are available. The ecoinvent LCI database includes over 100 datasets related to the primary production of different metals. However, many of these datasets have become outdated or lack detail. Here, we will report on an on-going project to update and expand, in a consistent, transparent, and flexible manner, the ecoinvent database with regards to the primary production of metals, focusing in particular on critical and scarce metals. Critical and scarce metals were evaluated with regards to the following aspects: (i) LCI dataset availability (in the ecoinvent database), (ii) ore types, (iii) geographic locations (mining and refining), and (iv) number of marketable products. Based on this analysis, a priority list of metals for the subsequent LCI data collection was established. A generic LCI data model for the primary production of metals was developed to have a high degree of modularity and to be (i) easily adaptable and applicable for all metals and (ii) duly reflective of the interconnectivity of metals production. Based on this model, we estimated the dataset requirements for the data collection phase. We found that even for a country-wise coverage of only 50% of the global production of all these metals, more than 300 inventory datasets would need to be compiled. Furthermore, significant data collection issues still remain; most notably related to difficulties in acquiring data from geopolitically unstable regions or due to the reticence of industry operators to disclose production data. Our presentation will give an overview of the actual status of the work in this important project; highlighting the international LCI data collection effort that is currently underway and discussing critical barriers to a more effective data collection.

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Comparison and improvement of life-cycle-modelling methods for recycling

Z. Jiang, H. Wang, Sichuan University / College of Architecture and Environment; W. Liao, Sichuan University / Institute of New Energy and Low-carbon Technology

Life-cycle modelling of recycling has been long a complex issue, and it has been difficult to find a widely accepted method to address the multifunctionality of recycling. ISO 14044:2006 has put forward basic, but inoperable, allocation procedures. Various allocation methods for recycling exist but have their own limitations and specific applicable scope. There has been no consensus on the most appropriate method. This study aims at offering a general model for comparison and improvement of different life-cycle modelling methods for recycling. A general schematic model is proposed to harmonise terminologies and parameters used in different methods. The model distinguishes between the mixed recycling route and the independent recycling route. Major existing methods, viz. the ILCD method, WRI 100-0 and 0-100 methods, PEF 50-50 method, and WSA method, are selected for a comparison. An improved method is developed to calculate the life-cycle-inventory value of a recycled raw material in the mixed recycling route. A case study of steel production involving mixed recycling of steel scrap (as the recycled raw material) and molten iron (as the virgin raw material) is conducted. The PEF 50-50 method was found to be most appropriate in terms of rules of interflow computation and attribution of recycling credits, applicable scope, and operability, as it attributes recycling credits to both the earlier and the later product systems, and has a broader scope of application. However, the PEF 50-50 method does not distinguish between the two recycling routes, and thus is not suitable for modelling life cycles involving the mixed recycling route. The electric arc furnace (EAF) route had higher potential environmental impacts than the basic oxygen furnace (BOF) route, which is not in line with our common understanding. Although the EAF uses less molten iron than the BOF route, its inputs of electricity, steel scrap, and iron alloy are higher; and the impacts associated with steel scrap is usually not considered. We compare five major existing methods for the life-cycle modelling of recycling and find that the PEF 50-50 method seems to be most appropriate. We develop the improved method based on the PEF 50-50 method and demonstrate its feasibility in the case study of steel production. The case study shows that environmental impacts associated with recycled raw materials are not always lower than that with virgin raw materials from the life-cycle perspective.

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The versatility of attributional LCA - a case study on recycling of Rare Earth Elements

D. Schrijvers, University of Bordeaux; F. Lartigue-Peyrou, Solvay Research and Innovation; G. Sonnemann, University of Bordeaux / ISM CyVi

Rare earth elements (REEs) recently gained interest in the domain of LCA. The market of REEs suffered a short crisis in 2011 due to export restrictions from China, who is the main producer of REEs. This has motivated the European Commission to identify several elements, such as neodymium, terbium, yttrium and europium, as critical. Yttrium and europium are building blocks of the material YOX, which is used in fluorescent lamps. Solvay has developed a process to recover YOX from waste powder, which is released after the conventional recycling of fluorescent lamps to recover mercury, glass and aluminium. We applied an LCA to determine the impacts of recycled YOX, compared to primary YOX. Several goal definitions had been formulated, which all aim to identify the responsibility for impacts of recycled YOX. Therefore, a cradle-to-gate attributional LCA has been applied. The goal definitions are related to different purposes of the LCA: environmental labelling or taxation, identification of hotspots and decision-making. Allocation at the point of substitution (APOS) had been applied to isolate the recycled YOX from the other co-products of the product system. This allocation procedure represents best our view on "responsibility for impacts", which we first formalized in an axiomatic scheme, as suggested by Heijungs (1998). Following the APOS method, first the inventory of the fluorescent lamp had to be calculated to identify what impacts of this product system are attributed to the recycled YOX. The impact assessment showed that recycled YOX is responsible for lower impacts than primary YOX. The inventory analysis enabled us to do additional analyses on the life cycle of the fluorescent lamp. The APOS methodology provided information that could not have been obtained by the cut-off approach or the substitution method, as it appeared that the powder could not be considered burden-free and a high recycled content of YOX in the lamp makes the lamp less responsible for impacts. Therefore, we conclude that attributional LCA can be applied for a wide range of LCA goal definitions, and the APOS method provided us with the most useful result in the current LCA. Heijungs R. 1998. Product innovation and eco-efficiency. Twenty-three industry efforts to reach the factor 4. Dordrecht, the Netherlands: Kluwer Academic Publishers. 296 p

Risk assessment and management of waterbodies (ground, fresh, marine and drinking waters)

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Active biomonitoring using the freshwater crustacean Gammarus fossarum: an operational tool to monitor chemical contamination and toxicity in continental surface waterbodies

G. Jubeaux, R. Recoura-Massaquant, BIOMAE; F. Massat, La Drôme Laboratoire; a. chaumot, O. Geffard, Irstea / UR MALY Laboratoire Ecotoxicologie

The active biomonitoring based on caged organisms is recognized as an alternative approach of risk assessment. Here, we propose to present *in situ* bioassays using the freshwater amphipod *Gammarus fossarum* with a standardized protocol to control field exposition and test organisms (one source population, calibration of size, sex, reproductive and physiological status, etc.). Based on studies conducted since two years among over one hundred stations of Water Framework Directive (WFD) monitoring French network, we will exemplify how this tool could be proposed as an alternative approach to assess and manage chemical contamination and biological status of waterbodies. On the first hand, we will show how *in situ* bioassays may be proposed to monitor priority substances of the WFD. Indeed, the Directive 2013/39/EU of WFD introduces Environmental Quality Standards (EQSs) for fourteen Priority Substances (PSs) in biota and some of those have been defined for crustaceans and molluscs. We conducted a large scale study for French Water Agencies between 2015 and 2016 where priority substances have been measured in gammarids exposed during 7 days in 140 stations. On the second hand, we will show in complement of regulatory obligations how the measurements of biological responses like feeding rate could be proposed to assess toxic impact of continental surface waterbodies. Ecotoxicological effects were assessed using caged gammarids. One of the employed toxicity marker was feeding inhibition, by mean of leaf consumption quantification. To interpret results with reliability, we use reference values integrating effects of environmental confounding factors (*e.g.*, temperature).

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Zebrafish larvae behaviour as a biological early warning system for aquatic systems

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An effective biological early warning system for the detection of acute contamination of aquatic systems should employ undemanding species that rapidly react to the presence of contaminants in their environment. The demonstrated reaction should be comprehensible and unambiguously evidential of the contamination event. Monitoring of zebrafish (embryo, larvae and adult) behaviour emerges as a new, sensitive method to study effects of compounds using a vertebrate model. Here, we propose to use behavioural changes of zebrafish larvae for the detection of sudden contamination events in a water stream. In Europe, early life stages of zebrafish are not protected as animals until 120 hours post fertilization (hpf), and hence zebrafish larvae at the age of 96 hpf were used to establish an animal-free vertebrate method for event detection. In order to employ the system not only for fresh water systems but also brackish water and marine systems, the influence of salinity on zebrafish larvae behaviour will be determined. In this study, zebrafish larvae were kept in formulated water until 96 hpf. Subsequently, they were transferred individually to a 96-well plate and exposed to a set of model compounds (cadmium chloride, permethrin, ethanol, sodium hypochlorite) in low concentration ranges and immediately monitored for avoidance behaviour which results in an increased activity. The presented methodology demonstrates an easily calculated and observable parameter for evaluation of the changes in zebrafish larvae behavior in response to acute exposure to sublethal doses of $CdCl_2$ and permethrin. The evaluation of the remaining tested substances will be conducted as well. The given proof-of-concept study aims at introducing a novel method for online water quality monitoring that utilizes vertebrates but is animal-free under EU legislation. The general applicability of the method is tested. The proposed method shows perspective in detecting chemicals already at low concentrations. To overcome ambiguity in the results and achieve higher precision, proper group size should be chosen. Since the system can be operated on a miniaturized scale, it is feasible to include larger groups.

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Vulnerability of surface and groundwater drinking water sources in the Netherlands to pesticides

R. Sjerps, KWR Watercycle Research Institute / Chemical Water Quality and Health; P. Stuyfzand, KWR Watercycle Research Institute / Geohydrology; L. Puijker, A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health

Pesticides could enter surface water and groundwater resources during and after application, which may imply a risk for the ecological environment or for human health. In this study we evaluated monitoring data of pesticides, including persistent and mobile recently authorized active substances, to signal and manage the risk of pesticides. In 60% of the 265 abstraction areas covering groundwater and surface water bodies in the Netherlands, pesticides have been detected. Around 100 pesticides and 14 metabolites were prioritized based on the extensive dataset (9,634 sampling sites over 2010-2014) according to their concentration above (half) the drinking water quality standard of 0.1 μg/L in raw water and drinking water (1 μg/L for human toxicological non-relevant metabolites). Dependent on the local land-use, soil and water system characteristics these compounds are relevant for uptake in monitoring programs. Examples of priority pesticides are bentazon, glyphosate, dikegulac sodium, bromacil, dimethomorf, oxamyl and glufosinate-ammonium. A selection of pesticides were studied for their behaviour in aquifers (results will be presented and will be available soon). A large part of the monitored pesticides (377) however have no priority for extensive further monitoring according to their concentrations below half the drinking water standard or below the detection limit. In addition, we signalled 48 mobile and persistent newly introduced pesticides on the market since 2005. A novel LC-MS/MS method for the simultaneous detection and sensitive quantification for 24 newly authorized pesticides on the market was developed and validated in drinking- and surface water. More than half of the compounds (15) were detected in 128 samples at locations susceptible for pesticide contamination in The Netherlands and in Belgium. Most compounds have been detected in the surface water samples; only two out of 90 groundwater samples contained recently authorized pesticides. In surface water, six of these pesticides already exceed the drinking water standard of 0.1 μg/L: the herbicide fluxapyroxad, the insecticides thiamethoxam and acetamiprid and the fungicides fluopyram, fluxastrobin and mandipropamid. The insecticide clothianidine, a neonicotinoid insecticide, was detected in groundwater above the drinking water standard of 0.1 μg/L. An evaluation regarding the risks for human health of the detected recently authorized pesticides will be presented.

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Metal accumulation and health risks for communities consuming Labeo rosae from the Olifants River, South Africa

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Globally, river ecosystems are among the most threatened habitats by human activities such as the release of chemical pollutants. The Olifants River system, located in the northeastern corner of South Africa with the lower part extending to Mozambique, is one the most polluted river system in Southern Africa. The Olifants River catchment is home to about 10% of South Africa’s population dominated by unemployed rural communities which rely on fish from this river system for protein suppliments. In the present study, metal levels were measured in the liver, gill and muscle, and the human health risk assessment was carried out to investigate if it’s save to consume *L. rosae* from Loskop and Flag Boshielo dams. At least 10 fish specimens were collected using gill nets in May/June and November/December 2014. Fish tissues were dissected out and frozen. The frozen samples were sent to the SANAS accredited water laboratory for metals analysis. Health risk assessment was performed following assumptions that 150 g of portion is consumed by a 70 kg adult with 30 years as an exposure duration. Metal concentrations exhibited liver>gill>muscle trend except for strontium which showed gill>muscle>liver. The metal concentrations and fish lengths have shown no relationship on all tissues at both impoundments. The non-metric multidimensional scaling has shown clear separation on the metal concentration for liver and gills between the two localities. Concentrations exceeding the international standard for safe consumption was observed for silver, arsenic, lead, antimony and selenium at both impoundments. Although eating fish is recommended globally due to its nutritional benefits, the consumption of *L. rosae* from the Olifants River system could result in adverse health effects.

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Contaminant fate and effects in marine environment: distance of the French seas from Good Environmental Status (MSFD - D8 - 2nd cycle)

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Under the Marine Strategy Framework Directive (MSFD, 2008/56/EC), Good Environmental Status (GES) of EU marine waters should be achieved by 2024. Among the 11 descriptors which describe what the environment will look like when GES has been achieved, the one corresponding to contaminants is the descriptor 8 (D8) and it states that "contaminants are at a level not giving rise to pollution effects". Monitored substances are contaminants *thati) are of environmental concerns: toxic, persistent, bioaccumulative and ii) for which ones standard methods and threshold values are available. The French D8 assessment approach and the resulting* environmental status of French marine environment regarding contaminants *will be here presented, as they are under preparation for the MSFD environmental assessment due to June 2017.* In France, a set of indicators has been defined upon the work carried out within the regional sea conventions (Ospar and

Barcelona). PAH, PCB, heavy metals (*e.g.* Cd, Pb, Hg) concentrations in molluscs and sediments were obtained from a 30-year French observatory program (ROCCH). PCB, dioxines, furans and heavy metal concentrations were assessed in different fishes collected in 2014 and 2015 during dedicated sea campaigns with combined fisheries and contaminants objectives. Imposex, as an OSPAR common indicator, was annually monitored on *Nucella lapillus* along the French Atlantic coasts since ca. 15 years. Sediment bioassays with oyster larvae were assessed in the Mediterranean Sea in 2009, 2012 and 2015. A panel of additional contaminant effects on fish and molluscs were assessed during dedicated sea campaigns in the Seine Bay in France between 2008 and 2012. This impressive amount of data is used to calculate the values of the indicators which are confronted to the corresponding existing thresholds (*i.e.* BAC, EAC, ERL, EQS). With sufficiently furnished time-series, trends are characterized using dynamic linear models, which is an approach tailored to environmental monitoring data characteristics (*e.g.* irregular sampling frequency, missing data). Outcomes of this massive assessment will thereafter be aggregated according to a method developed under ICES/OSPAR in order to assess distance from GES regarding contaminants in France in 2018.

Do we have the right tools to identify emerging hazards and risks? (I)

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WHO Chemical Risk Assessment Network - new and emerging risks
R. Brown, World Health Organization
The United Nations Conference on Sustainable Development held in Rio de Janeiro, Brazil in 2012 ("RIO+20") called for effective systems for the sound management of chemicals throughout their life cycle, including to respond to emerging challenges. Chemicals can cause both acute events ("poisonings") and also non-acute, long-term health effects. Surveillance systems can identify acute events with known chemicals, but surveillance also has the potential to identify new threats to health, known threats with a changing pattern of occurrence and emerging risks from chemicals with a less acute onset. Poisons centres can have a critical role to play in this process. The World Health Organization (WHO) works with countries to strengthen their capacities for the sound management of chemicals. This includes core public health capacities for detecting chemical events, which are a requirement for countries under the International Health Regulations (2005). Examples of chemical events identified through these mechanisms will be presented. However, many countries do not yet have adequate capacity, even for detection of acute events from known hazards. To strengthen capacity for management of chemicals and risk assessment, WHO maintains the WHO Chemical Risk Assessment Network, which covers more than 70 institutions in 40 countries. One objective of the Network is to assist in the identification of emerging risks to human health from chemicals. A Network activity has been proposed for the signalling of new and emerging risks. Through the Network, this activity has the potential to extend beyond Europe through institutions involved with chemical risk assessment globally.

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A study preparing for a strategy for a non-toxic environment, according to the 7th Environmental Action Programme of the European Parliament and of the Council
U. Boije af Gennäs , European Commission - DG Environment
Aim \n This presentation will provide a background and present highlights of the final report prepared as part of the EU 7th Environment Action Program on the 2018 strategy for a non-toxic environment. The study was commissioned by DG Environment (European Commission) and conducted by the contractor Milieu and the sub-contractors Risk Policy Analysts (RPA), Ökopol and RIVM. The study focuses on seven topics being substitution, substances in articles and non-toxic material cycles, protection of children and vulnerable groups, very persistent chemicals, innovation, development of green chemicals and early warning of approaching chemicals threats. \n *Methods* \n For each topic, the study includes a literature review, describing the health and environmental issues, the current state of play of policy, gaps and deficits as well as improvement opportunities and best practices. Further, a workshop was held in Brussels on 8-9 June 2016 to collect input from global experts and stakeholders, in particular on improvement opportunities and experiences from past and ongoing activities. Interviews and questionnaires were also used in the study. \n *Results* \n The results of literature review, workshop and other collection of facts and views are presented in the final report. Apart from descriptions of the status quo for the different sub-study topics, it includes listings of improvement opportunities to address the identified gaps and deficits as well as best practices. It also contains recommendations to set up a European Early Warning System. Highlights of the final report are selected and will be presented. \n *Conclusions* \n A preliminary conclusion is that there is a large interest in the topics covered within academia and among different kinds of stakeholders both in Europe and beyond. The level of activity in looking for and trying out different kinds of solutions to the problem involved is also considerable. There also seem to be strong interconnections and synergies between different measures to address gaps and deficits identified.

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Identification, prioritization and evaluation of potential New Emerging Risk of Chemicals (NERCs) for the environment
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New and emerging risks of chemicals (NERCs) continue to be reported despite existing laws and regulations to limit the risks of dangerous substances in the environment. Existing laws and regulations often address chemicals with extensive evidence on harmful effects. As a consequence there is a large time span between the first indication of the presence of a substance in the environment and actual measures taken to control the risk posed by these chemicals. To be able to react in a proactive manner there is need for a system that identifies NERCs as soon as possible and map out strategies or possibilities to manage the identified risks to the environment. An integrated approach was used for the early identification of NERCs by the collection of signals from news reports, periodic screening of literature and websites, expert consultation and analytical environmental screening studies. The signals are valued on potential emerging risks by a tiered approach applying selection criteria and a prioritization scheme. Potential NERCs were identified and prioritized by means of an impact analysis. This analysis is based on a first assessment of the severity of the hazardous properties and the likelihood of exposure. Potential NERCs are categorised into five risk priority classes. Information on hazardous properties and exposure is collected from public databases. If the required information is not available, the hazardous properties and exposure are estimated by applying for instance QSARs and exposure categories based on the characterisation of the main type of use and the production volume. Before the data collection starts current measures that are already being taken regarding registration, classification, authorization and restriction, etc. are checked to see whether the identified risk is already being addressed The methodology for selection and prioritization of NERCs has intensively been applied and tested. The prioritisation scheme for instance has been applied to the results from two analytical screening studies providing a set of chemicals that were identified in fresh surface water samples and wastewater treatment effluent. This resulted in set of substances that deserve the most attention and finally led to the selection of some chemicals for further risk management option analysis under REACH.

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Establishment of the European Exposure Strategy 2025: Common interests of SETAC Europe and ISES Europe for picking up, sharing and communicating signals
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With about 650 participants from 50 countries, the ISES (International Society for Exposure Science) Annual Meeting 2016 in the Netherlands was the biggest since the start of ISES in 1989. The need to have a European programme stimulating increased generation of exposure information followed by independent assessment and use by risk managers and policy makers was felt throughout the meeting. This enthusiasm resulted in the establishment of ISES Europe as Regional Chapter to ISES, just two months later. ISES Europe is regarded to be crucial in starting off such a programme in Europe as at the moment no other independent organisation is likely to be able to do this job. ISES Europe's objective is to facilitate generation and sound use of exposure information to improve protection against environmental, consumer and workers health risks. ISES Europe aims at the development of an infrastructure to share, construct and disseminate exposure information as well as underlying knowledge and methodologies. In Europe, current information needs are largest for chemicals. EU chemicals frameworks are complex and critically important, also for industry and commerce in many other countries. Therefore, the initial focus of ISES Europe will be on chemicals. At a later stage, physical and biological stressors may be incorporated. The wide availability of chemical production and exposure data in Europe (e.g. ECHA website) made that health and safety issues of chemicals have become a business issue for CEOs. In the public domain, the policy move towards a non-toxic environment in 2050 has set additional challenges for a transition towards a circular and bio-based economy and the use of green chemistry. Generation of any data - hazard data or exposure data - should not be a free choice. Anchoring of these into regulatory requirements and guidance is pivotal to create a level playing field. Truly based on participation of academia, research funders, insurance companies, workers unions, CEO's, competent authorities and societies like SETAC Europe, ISEE Europe and EUROTOX, ISES Europe will play an essential role in this via the development of a European Exposure Strategy 2025. Crucial are education and training, a formal certification, scientific networking, the EU research agenda and facilitating interdisciplinary collaboration and exchange. During the presentation, more details will be presented as a kick off for the plenary debate at the end of the session.

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Panel discussion

21st century Ecotoxicology and Human toxicology: Applications and perspectives for the use of OMICs data (II)

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Peptidomics: a new omics-sibling in ecotoxicology?

S. Husson, University of Antwerp / Biology; K. Van Camp, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Biology; G. Baggerman, Flemish Institute for Technological Research VITO / Department of Biology SPHERE; R. Blust, Systemic Physiological and Ecotoxicological Research SPHERE University of Antwerp / Biology

(Neuro)peptides are small messenger molecules that are derived from larger, inactive precursor proteins by action of processing enzymes. These biologically active peptides can be found in all metazoan species where they orchestrate a wide variety of physiological processes. Obviously, detailed knowledge on the actual peptide sequences, including the potential existence of truncated versions or presence of post-translation modifications, is of high importance when studying their function. A peptidomics approach therefore aims to identify and characterize the endogenously present peptide complement of a defined tissue or organism using liquid chromatography and mass spectrometry. In a first attempt to biochemically identify endogenously present peptides from brain tissues of the zebrafish *Danio rerio*, a peptidomics workflow was employed. The entire brain region of 6 male adult zebrafishes were carefully dissected and 6 independent (neuro)peptide extracts were made using an extraction protocol that is extremely efficient in avoiding the presence of protein degradation products. The 6 peptide samples were analyzed using a nanoLC instrument that is directly coupled with an LTQ-Orbitrap mass spectrometer to yield biochemical identifications of 62 peptides that belong to 34 different precursor proteins. In addition, a remarkable amount of shortened forms (aminoterminally or carboxyterminally truncated) could be identified, yielding a total number of 105 peptide identifications. To our knowledge, this is the first high-throughput peptidomics study of a fish species. As such, this archive of identified endogenous peptides is likely to aid future research in (neuro)endocrinology in this important model. However, it is very important to realize that the endogenous peptide content of a cell, tissue or organism is spatially and temporally dynamic. Furthermore, remarkable inter-individual variations exist, even when using specific zebrafish strains. Taking these pitfalls together, application of in-depth differential peptidomics technologies in an ecotoxicological research setting e.g. to study modulatory effects of defined exposure scenarios on neuropeptidergic signalling systems is very challenging. Nevertheless, this pioneering study can be considered as a basic blue print of endogenously present peptides in the fish brain, which is indispensable for further attempts to monitor changes in peptide expression in response to changes in the organism or the environment.

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WHEN PROTEOGENOMIC HELPS ECOTOXICOLOGY

C. Almunia, CEA; D.D. Gouveia, Irstea Lyon / UR MALY Laboratoire Ecotoxicologie; J. Trapp, Irstea / Laboratoire décotoxicologie; Y. Cogne, O. Pible, CEA; a. salvador, Université Lyon1; a. chaumot, O. Geffard, Irstea / UR MALY Laboratoire Ecotoxicologie; J. Armengaud, CEA / Laboratory Innovative technologies for Detection and Diagnostics

Because of their ecological representativeness, invertebrates are commonly employed as test organisms in ecotoxicological assessment. Among them, some non-model species are used as aquatic “sentinel species” to examine the water quality. To date, biomarkers employed for these species were the result of a direct transposition from vertebrates, despite deep evolutionary divergence. To gain efficiency in the diagnostics of ecosystem health, specific biomarkers must be developed. With shotgun proteomics, proteogenomics and “state of art” mass spectrometers, the discovery of proteins and signatures of environment pollution has become possible and applicable to routine ecotoxicological assessment. Here we describe how proteomics remains a challenge for ecotoxicological test organisms because of the lack of appropriate protein sequences databases, thus restricting the analysis on conserved and ubiquitous proteins. These limits and some strategies used to overcome them are discussed and illustrated by the investigation of male Gammarus Fossarum, a freshwater Crustacean in response to endocrine disruptors. These new tools, such as proteogenomics and targeted proteomics, should result in new biomarkers specific to relevant environmental organisms and applicable to routine ecotoxicological assessment.

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Proteome modulation as an early marker of gamma irradiation effect on Caenorhabditis elegans egg hatchability?

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Non-human species are subjected to ionizing radiation chronic exposure, but involved toxicity mechanisms remain unclear. In addition for a same cumulated-dose the observed effects are different after acute and chronic-radiation meaning differential underlying mechanisms. After a huge acute dose of gamma rays, proteome protection pathway particularly against oxidation such as carbonylation, can be linked to differential radiosensitivity (Krisko 2010). However, to date there is no data about protein carbonylation (PC) after chronic exposure and after acute at lower doses than 200Gy. The aim of this work was to better understand the role of both PC *i.e.* content and nature, and proteome changes in the acute *vs* chronic effects induced by gamma irradiation. *C.elegans* were exposed to different acute doses of γ rays (^{137}Cs) *i.e.* from 0 to 200Gy. The cumulated dose 2.5Gy was studied both by chronic and acute exposure. After irradiation, reproduction was monitored by measuring the number of offspring per individual and the rate of hatchability. Samples for CP and proteomic study were subjected to a protein extraction, carbonyls derivatization and protein separation by two dimensional electrophoresis followed by signal analysis. Spots of interest were analyzed by mass spectrometry (MS). A significant decrease of offspring number per individual was observed after both chronic and acute exposure from 2.5Gy and 30Gy, respectively. In addition, conversely to chronic exposure a decreased rate of hatchability was observed after acute irradiation from 30Gy. Results of carbonylomes revealed 113 different carbonylated protein spots between controls and exposed after acute irradiation whereas only 29 differ after chronic irradiation. MS revealed that most of the identified proteins are involved in the reproduction of *C. elegans*. These results are particularly interesting as three of the common proteins between both irradiation modes, required for embryo and spermateca development (Elongation factor 2, mRNA transport homolog 4, actin 4) are up-carbonylated after acute exposure or over expressed after chronic exposure.These results highlight that the main differences are at the level of germline development proteins that could be an early marker of effect. A 2D-DIGE methodology comparing both modes (from 0 to 3Gy) will help to refine this hypothesis. If trends are confirmed protein modulation could become a new biomarker to assess radiation-induced toxicity molecular mechanisms.

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Developmental neurotoxic effects of pesticides, MeHg, and PFHxS combining behavior and cognitive studies in mice with metabolomic pathway analysis

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Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The EU-funded project DENAMIC investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. In the current study the aim was to investigate the behaviour and cognitive effects of pesticides, methylmercury, and perfluorohexane sulfonate (PFHxS) exposure in mice and to further study the underlying molecular mechanisms of the observed effects using metabolomics and proteomics. Male mice were exposed to a single dose of pesticides (chlorpyrifos, carbaryl, cypermethrin, endosuldfan), methylmercury or PFHxS at postnatal day 10. Behaviour effects were studied at the adult age of 2 to 4 months. The studies showed that all chemicals can cause developmental neurotoxic effects, even after a single exposure which was given at a vulnerable period of brain development. The cognitive and behaviour tests showed that the early life exposure of the compounds can alter adult spontaneous behavior and cognitive function. Interesting the chemicals had different modes of action causing the same apical endpoint (increased spontaneous behaviour). Targeted and untargeted metabolomics and proteomics studies were carried out in brain tissues of cerebral cortex and hippocampus. Biochemical networks were mapped for each brain tissue using MetaMapp and Cytoscape. Metabolomic and proteomic analysis showed that a limited number of metabolites and proteins were down or upregulated, and that most were regulated in hippocampus. The strongest effect was found for PFHxS. Regulated metabolites and proteins were mostly related to axons/neurons, mitochondria, purine pathway, NADPH/NAPD activity, ATP/ribonucleotide metabolic processes. In general, the results showed that the cholinergic system was affected. A positive correlation was found between the levels of the neurotransmitter acetylcholine and acetylcarnitine in hippocampus; it has been suggested in literature that an increase of acetylcarnitine can give a spontaneous release of acetylcholine. The developmental toxic effects might cause neurological/neurodegenerative disorders/diseases.

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Multi-Omic approach to metabolic disruption: transcriptomic and metabolomic integration to analyze the effects of bisphenol A in zebrafish embryos

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One of the major challenges of omic technologies is to translate complex datasets into testable predictions of phenotypic adverse effects. In this work we combined

high throughput RNA sequencing data with high quality, non-targeted LC-MS metabolome analyses to investigate both metabolomic effects and the subjacent regulatory pathways of exposing 5-day zebrafish embryo to bisphenol A. The results show that even a moderate coverage of the zebrafish metabolome (from 50 to 100 metabolites) may be representative for almost all affected major metabolic pathways, and that the changes on metabolite concentration were not directly predictable form transcriptomic data. This type of analyses, which involved multivariant chemometric models as well as standard tools for transcriptome analyses, can easily incorporate other non-targeted omic approaches, including lipidomics, proteomics, and/or epigenetic data at the genomic scale, among others. We conclude that only a combination of non-targeted omic approaches can correctly predict macroscopic adverse outputs from molecular data, a paradigm for predictive (eco)toxicology. *Acknowledgements* - This work was funded by the Spanish Ministry of Science and Innovation (CTM2014-51985-R) and by the Advanced Grant ERC-2012-AdG-538320737 from the European Research Foundation. LNM acknoledges a Beatriu de Pinos Postdoctoral Fellowship (AGAUR-MSCA Cofund-2013BP-B-00088).

Environmental endocrine compound concentrations and human and ecosystem health effects

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Assessment of chronic effects of chemicals and detection of potential endocrine-disruptors for a hermaphrodite mollusk: possible endpoints in a full life-cycle bioassay.

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For several years, the need for life cycle tests that are sensitive to chemicals and that can help detecting endocrine-disrupting chemicals (EDCs) is recognized. Partial life-cyle tests measuring apical endpoints such as fecundity that can respond to both EDCs and some non-EDCs have been recently published for freshwater molluscs. In its Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals, OECD includes these tests as a level four test i.e., a test with apical endpoints demonstrating adverse effects on whole individuals, but with little or no mechanistic value. Indeed, until now, endocrine or neuro-endocrine underlying mechanisms of reprotoxicity in molluscs are not known. For the terrestrial environment, few life cyle tests are available. The aim of the present study was to assess the chronic effects and to detect potential ED effects of chemicals on a simultaneous hermaphrodite soil organism, based on the measurement of apical endpoints (hatchability, survival, growth, fecundity, fertility, histopathology of gonads). A full lifecycle (240 days) bioassay using the terrestrial snail, *Cantareus aspersus*, allowing exposure from embryogenesis to reproduction, was used to assess the effects of Bypass®, a glyphosate-based herbicide (GlyBH). A mixture (R-A) made of diquat (Reglone®) and nonylphenols (NP, Agral®), known for its ED effects in other organisms, was also tested. At environmental concentrations, both pesticides enhanced growth but disrupted reproduction without any visible effects on gametogenesis. The R-A mixture strongly reduced the number of clutches, possibly due to a permanent eversion of the penis, suggesting a disrupting effect at the neuro-endocrine level which prevented normal mating. Bypass® had contrasted endocrine disruption effects, with a significant enhancement of growth speed followed by a marked reduction of the fertility of *C. aspersus* snails. The hormetic effect of GlyBH on growth could results of a modulation of the cell activity of the mesocerebrum involved in growth regulation whereas the decreased fertility of snails exposed from the embryogenesis could imply an alteration of the quality of eggs laid or/and of their fertilization. The presented full life-cycle bioassay provides original data on chronic effects of chemicals and offers new opportunities to progress in the challenging identification of endocrine perturbations in hermaphrodite organisms.

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Steroid and thyroid gene transcription ontogeny during the first 32 days of zebrafish development improves our understanding of endocrine disruption

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Effects of endocrine disruptors (EDCs) are usually well described at the morphological level, but underlying mechanisms are still poorly understood. Transcription patterns of steroid and thyroid genes during normal development can be used as a reference dataset. By combining this information with knowledge on morphological effects of EDCs, we can hypothesize and validate molecular mechanisms of endocrine disruption. Studies using zebrafish embryos reported a wide range of effects of 17α-ethinylestradiol (EE2), including skeletal malformations, pericardial and yolk-sac oedema and an uninflated swim bladder. First, we described the timing of the normal embryonic transcriptional activation of the thyroid and steroid hormone synthesis machinery and associated receptors. We isolated RNA at 25 time points between 0 and 32 days post fertilization of zebrafish development. mRNA levels of 20 genes involved in the steroidogenic pathway and 9 genes related to the thyroid metabolism, were measured by QPCR. Second, we created an overlay of the ontogeny data of the estrogen receptors and vitellogenin 1 with morphological effects after EE2 exposure in order to identify specific transcriptional events that are associated with adverse developmental effects. These associations will be formulated as working hypotheses, which will be validated in a next set of experiments. We observed different transcriptional patterns of estrogen receptors during development. E*sr2a* is maternally transferred. While *esr1* is abundantly transcribed around the time of embryonic genome activation (6 hpf), transcription of *esr2a and esr2b* increases later in development. Transcription of *vtg1* shows peaks which correspond to elevated transcription of *esr1.* In exposures to EE2, we observed dose-dependent skeletal malformations as early as 54 hpf, compared to the controls. Our results indicate an increase in transcriptionof *esr1* and *vtg1* in the period of embryonic genome activation (~3 hpf). These data highlight the possible importance of *esr1* in the estrogen signalling transduction in the early developmental period. These ontogeny results will improve our fundamental understanding of the role of steroid and thyroid hormones during early life stages of the zebrafish and will be applicable to the zebrafish research community. Furthermore, the approach of combining ontogeny data with exposure data will facilitate a more thorough understanding of the molecular mechanisms of endocrine disruption.

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Combined exposure to EDCs resulting in neurodevelopmental disorders

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It has been well established that organic compounds such as phenols, PCBs phthalates exert endocrine disrupting activity, however, the contribution of heavy metals is more questionable. In the current study, phthalate and heavy metals (Pb and Hg) prenatal exposure was determined measuring 11 phthalate metabolites in urine, Pb in blood and Hg in hair of (n=149) mothers during the third trimester of pregnancy (prenatal exposure) and from their children at the 24th month of age (postnatal exposure). Urine untargeted metabolomics analysis was also carried out in a Thermo Orbitrap LC/MS-MS. Psychomotor development was assessed in children at the age of 2 years by the Bayley Scales of Infant and Toddler Development. Associations were investigated using the linkage disequilibrium method of EWAS, while pathway analysis was mapped with the Mass Profiler Pro (Agilent Technologies). Exposure levels to both phthalate metabolites and metals were far below the respective biomonitoring equivalent values, while from the EWAS analysis, it was found that child cognitive development was inversely associated with natural log concentrations of metabolites of DEHP, BBzP and DiNP in the urine, as well as the Pb in blood and the Hg in hair collected from mothers. With regard to post-natal exposure statistically significant association was the inverse correlation of Hg in hair and cognitive functions for females. Metabolic pathway analysis revealed that alterations in urine metabolites are related to the TCA cycle, suggesting impaired mitochondrial respiration; the latter is central to energy metabolism and cellular signaling and plays fundamental roles several cellular processes. Inhibition of mitochondrial oxidative phosphorylation could also cause a defective mitochondrial energy production during the process of fetus formation and development that are reflected in early life motor development. The latter is enhanced by the oxidative stress induced by heavy metals. This defective mitochondrial energy production during the process of fetus formation and development is reflected in early life motor development. The key finding of the study is that although phthalates and metals affect mitochondrial respiration through different mechanisms (endocrine disruption and oxidative stress respectively), this synergistic effect is essential for the deployment of neurodevelopmental defects.

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Using cats as model for indoor exposure of thyroidogenic compounds

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Household pets such as cats spend a significant time indoors. This and in combination with their intense cleaning of their fur by licking, makes them particularly exposed to house dust and chemicals associated to the dust. Cats are exposed to dust through inhalation but more importantly through ingestion. Recently it has become evident that dust is a significant source of exposure to POPs such as PBDEs, and an association has been demonstrated between domestic cat's thyroid health status and levels of brominated flame retardants in their blood. Thyroidogenic disruption is of growing concern for human health and the environment. Hence, the MiSSE project (Mixture aSSessment of Eendocrine disrupting compounds), is specifically aiming to assess the indoor exposure situation to thyroid hormone disrupting compounds (THDCs), and accordingly the mixture effects of these compounds. As cats and toddlers have a similar behavior with their grooming and hand-to mouth activity cats are here used as sentinels for human and child exposures to indoor related chemicals and their thyroidogenic effects. Here we will report the results of the chemical analysis of paired samples of cat serum and household dusts from 17 households in Sweden. Cat serum was analyzed for brominated flame retardants, organochlorines, phenolic compounds, and perfluoroalkyl substances (including the phosphates), whereas dust was also analysed for the phthalates and organophosphorous compounds. Correlations between cat serum and house dust in paired samples were assessed. The study showed that cats internal exposure is reflecting the external dust associated exposure of a range of organohalogen compounds. It is intriguing to see that compounds already regulated, such as BB-209, DEHP and TCIPP are dominating the chemical profile in dust. Whereas the PFOA and PFOS was measured at highest levels in the cat serum, exceeding the PBDEs with a factor of 100. The metadata collected with the questionnaire is not yet fully evaluated, but in general it can be concluded that these compounds are ambigous in our indoor environment and that it is difficult to identify parameters that would influence the levels found in the household dust. The chemical mixture identified in dust and cat serum have been evaluated for its thyroid hormone disrupting potency and will be presented at SETAC Europe 2017 by Jessica Legradi (zebrafish studies) and Timo Hamers (in vitro studies).

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Exposure to persistent organic pollutants and risk of Metabolic Syndrome in the population of Catalonia
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Persistent organic pollutants (POPs) are man-made chemical compounds usually produced for agricultural and industrial applications, and possess notable toxicity potential and persistence, hence posing a threat to humans and the environment. Exposure to POPs has been related to increased risk of some diseases such as diabetis and most recently, Metabolic Syndrome (MetS). Metabolic Syndrome is a group of risk factors characterized by central obesity, dyslipidemia, glucose intolerance and arterial hyperthension. The relationships between POPs and MetS are, however, not well established yet. Moreover, the relationships between POPs and lipid-related factors such as the body mass index or blood cholesterol and tryglicerides can unmask the real associations between concentrations of POPs and MetS. In this sense, Structural Equation Models (SEM) allow to take into account such confounding factors that may affect the resulting associations. This research uses Structural Equations Modelling to assess the prevalence of Metabolic Syndrome in a Mediterranean general population (that of Catalonia, in Southern Europe) against a set of persistent organic pollutants, including organochlorine pesticides (OCPs), polychlorobiphenyls (PCBs) and polybromodiphenyl ethers (PBDEs). The model is additionally adjusted by several covariates such as age, sex, social class, educational level, physical exercise, smoking habit and alcohol consumption. One organochlorine pesticide (hexachlorobenzene) and one polychlorobiphenyl (PCB-118) have been found to be strongly associated with Metabolic Syndrome. Also the OCP beta-hexachlorocyclohexane shows an association with MetS, but to a lower degree (90% of probability). This is the first study from a Mediterranean population showing that chronic exposure to a mixture of POPs can increase the risk of Metabolic Syndrome.

Behavioural ecotoxicology: Unravelling behavioural responses to aid environmental and regulatory toxicology (II)

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Larval VTG: reliable biomarker or deceptive molecular cue?
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The complex nature of crude oil contributes to highly variable toxicological effects in fishes, and a select few of these effects have been well characterized. Though the impact of developmental exposure on later life stages is less commonly studied, it is equally important. Studying the effects of embryonic oil exposure on ecologically relevant behavioral endpoints in adult fish helps to further characterize how oil spills affect fish populations. Male aggressive-based behavior is an ecologically relevant endpoint as it plays a critical role in the growth and reproductive success of fishes. Changes in aggressive behavior in fish could be due to endocrine disruption and exposure to estrogenic compounds. In our study, we attempted to link larval gene expression of the yolk sac precursor protein vitellogenin (VTG), a commonly used biomarker of exposure to estrogenic compounds, to male aggression during later life stages. We developmentally exposed sheepshead minnow (*Cyprinodon variegatus variegatus*) to water accommodated fractions of three different oil types (a source oil and two oils varying in amount of weathering) as well as a dispersant from 1-10 days post fertilization (dpf). Expression levels of VTG were measured immediately post-exposure at 10dpf. The fish were then grown up in clean water and assessed for aggression-based behavior at 8-11 months old by quantifying attacks of their reflection. We found that larval VTG expression post-embryonic oil exposure does not correlate with male aggressive behavior in adult sheepshead minnow. Studying ecologically relevant behavior such as male aggression in fishes enables more thorough and accurate predictions of the consequences of oil exposure on fish populations. However, molecular biomarkers in larval fish should be used with caution, as they may not be indicative of effects seen during later life stages.

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High throughput sequencing and behavioural endpoints highlight effects of antidepressants on crustaceans at environmentally relevant concentrations
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The effects of antidepressants on wildlife are currently raising some concern due to an increased number of publications indicating biological effects at environmentally relevant concentrations (< 100ng/L). These results have been met with some scepticism due to their experimental designs, the higher concentrations required to detect effects in some species and the perceived slowness to therapeutic effects recorded in humans and other vertebrates. Previously we have demonstrated that environmentally relevant (1-100ng/L) concentrations of fluoxetine and several other antidepressants can effect behaviour in scototaxis/phototaxis and activity based experiments. Here we present the results of several repeated behavioural trials exposing gammarid amphipods (*Echinogammarus marinus* & *Gammarus pulex*) to antidepressants linked to gene expression using next generation sequencing. The animals were exposed to these five antidepressants at environmentally relevant concentrations from 1 to 1000 ng/L during short-term (1 hour and 1 day) and medium-term (8-14 days) experiments. The movement of the amphipods was tracked using the behavioural analysis software during 12 min alternating dark/light conditions. Antidepressant concentration had a significant effect ($p < 0.01$) on velocity for Duloxetine (1hr, 1day & 8days); Sertraline (1hr & 1day) and Fluoxetine (1day) but not trazodone or citralopram ($p > 0.05$). We have also applied high-throughput sequencing technology to animals exposed to fluoxetine at 1, 10 & 100ng/L concentrations of fluoxetine to reveal the broad transcriptomic responses to these compounds. HiSeq sequencing generated approximately 15million reads per sample and those that were exposed to fluoxetine could be clustered distinctly from controls. The number of distinct annotated proteins (uniprot) that differed significantly from the control increased in a concentration dependant manner from approximately 30 (1ng/L) to 60 in 100ng/L. The overwhelming pattern of gene expression relates to heat shock (stress proteins) and glucose metabolism and plus several indicators of neurological modulation. Amphipods exposed to SSRIs display changes in multiple behaviours easily quantified using behavioural analysis software. These individuals display altered gene expression profiles after 1 week's exposure to SSRIs with plausible changes in pathways relating to serotonin modulation.

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Persistent Organic Pollutants chronic dietary exposure disrupt behavioral and molecular responses over several generations in unexposed zebrafish (Danio rerio)
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Polychlorinated Biphenyls (PCBs) and Polybrominated Diphenyl Ethers (PBDEs) are Persistent Organic Pollutants (POPs) extensively used during the 20th century, especially as additives, stabilizers or flame retardants. Despite their total or partial ban for being either produced or used, these pollutants are still present in many environmental compartments. Harmful effects of POPs, in particular behavioral disruptions, have been shown in several species both in directly exposed animals and their offspring. At 5 days post fertilization, zefrafish larvae were exposed chronically to a PCB-PBDE mixture *via* diet and chronic exposure lasted during all life stages. The mixture contains 22 PCB congeners and 7 PBDE congeners, chosen because of their presence in sedimentary compartment and with proportions and concentrations close to environmental situation (**ΣCB**= 1974.48 ng g-1 and **ΣBDE**= 488.90 ng g-1 wet weight in food). POP-exposed fish produced offspring (F1, F2 and F3 generations) which were fed using plain diet. Behavior, including larval photomotor response (PMR, F1 to F3) and novel tank diving test at 3 months (F0 to F3), gene expression (F1 to F3, DNA methyltransferase and *c-fos)* and whole brain monoamines analyses (F0 to F3) were performed to explore potential neurobehavioral disruptions and epigenetic mechanisms. On one hand, F0 and F3 generations were not clearly impacted by chronic POPs exposure. On the other hand, genes expression variations were observed in both F1 and F2 generations and were correlated with larval PMR responses. Moreover, anxiety-like behavior was observed during novel tank challenge in F1 and F2 generations Brain-monoamines assays are still pending and differential levels in different generation could support our findings. *In natura*, the behavioral disruptions observed could impede access to food, exploration of new territories or reproduction and hence contribute to exposed population size decrease. French National Research Agency funded project Fish'n'POPs, ANR-13-CESA-020.

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Pharmaceutical alters mate choice and gene expression of a fish
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Aquatic habitats are increasingly being exposed to chemicals that, even at low levels, can disturb the endocrinology of organisms. Among the handful of studies that have examined the behavioural effects of endocrine disrupting chemicals (EDCs), none have attempted to link the genetic mechanisms underlying behavioural changes with a systematic investigation of their ecological effects. New technologies, such as next-generation sequencing (NGS), offer a powerful toolkit to uncover the molecular pathways related to chemical pollutants. Accordingly, the aim of this study was to investigate the impacts of short-term (24-day) exposure to an environmentally relevant concentration of EE2 (5ng/L), a synthetic estrogen used in the contraceptive pill and a widespread contaminant of aquatic systems globally - on male mate choice and gene expression in the guppy. To investigate the impact of EE2 on visual and chemical communication of guppies a male was presented with a choice between (1) the visual cues of an exposed and a control female, and the following day (2) the odour cues of an exposed and a control female. To determine how gene expression related to behaviour of exposed individuals, total RNA was isolated from the whole brain tissue of all males and females used in the behavioural assays. Samples were sequenced using Illumina HiSeq 2000 platform 100bp paired-end, strand-specific reads. We found that EE2-exposed males spent significantly more time associating with exposed females when they could only use visual cues. By contrast, when they were given only chemical cues to choose from, EE2-exposed males visited control female chemical cue more often than EE2-female. Interestingly, based on the differential gene expression analysis, we found unique gene transcripts that were either induced or repressed due to EE2-exposure, or were sex-specific and showed biased abundance in female or male libraries. Our study shows that exposure to EE2 alters male mate choice and female behaviour in the guppy. Not only does our study uncover a previously unknown behavioural impact of EE2-exposure on female behaviour, but highlights unique gene transcripts that were either induced or repressed due to EE2-exposure, or were sex-specific and showed biased abundance in female or male libraries. Our results, among the first to link behaviour and gene expression, will highlight how chemical pollutants impact individuals at multiple levels.

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Avoidance behavior: the weight in recovery and resilience of ecosystems under salt stress
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Climate change scenarios point to an increased risk of seawater intrusion in coastal freshwater ecosystems. The assessment of the ecological risks due to seawater intrusion is usually supported on available standard protocols that neglect the ability of mobile organisms to avoid salinized environments. Avoidance has been shown to be a relevant endpoint to be included in ecological risk assessments, since: i) avoidance may be considered as important as mortality and ii) avoidance has been previously reported as an earlier response to stress. This work intended to assess the integration of this endpoint in ecological risk assessment schemes of ecosystems under saline stress. For that, the following specific objectives were delineated: i) to assess the avoidance response of freshwater species to increased salinity, and ii) to compare avoidance sensitivity with results obtained from standard toxicity assays (e.g LC_x or EC_x). Avoidance behavior to a gradient of salinity was studied for the following species: *Daphnia magna*, *Danio rerio*, *Heterocypris incongruens* and *Xenopus laevis*. The salt NaCl was used as a surrogate of natural seawater to create the saline gradient. All exposures were run for 12 h in total darkness. Regarding the second specific objective, data sets obtained for LC_{50} values and AC_{50} values were plotted in Species Sensitivity Distribution curves (SSD) and the Hazard Concentrations (HC_5)werecomputed and compared. Results showed when species are confronted with a saline gradient,are are able to escape at concentrations lower than those inducing lethal effects. The avoidance assays showed that *D. magna* was the most sensitive species with an AC_{50} value of 4.99 $mScm^{-1}$. The LOEG values were 17.14, 4.37 and 4.51 $mScm^{-1}$ for *D. rerio, D. magna* and *X. laevis*, respectively. Regarding the comparison of SSD curves, *D. magna* showed the highest difference between LC_{50} and the AC_{50} (4.37 and 11.68 $mScm^{-1}$, respectively). Computed HC_5 from SSD curves were of 7.34 (4.49-12.0), 3.43 (1.66-7.06) and 2.33 $mScm^{-1}$ (0.67-8.09) for LC_{50}; AC_{50} and LOEG, respectively. The escape of organisms at low salinity levels than those causing lethal effects, may result in disturbance of the energy transfer among trophic levels and consequently in the equilibrium of ecosystems. Results here presented corroborated the increased importance of conducting avoidance assays with aquatic organisms to increase the accuracy of ecological risk assessment on saline stress within a context of climate change.

Predictive models in ecotoxicology: bridging the gap between scientific progress and regulatory applicability (II)

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Use of TK/TD models in pesticide risk assessment
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Risk assessment for pesticides used in agriculture addresses the risk for many no-target organisms. It is regulated by Regulation 1107/2009 [1] that deals with data requirements and uniform principles. Additionally, SANCO and Efsa guidance documents provide all technical and scientific elements, and risk assessment schemes. They are all based on a step-wise approach, from basic worst case approaches to high-tier risk assessments based on more complex and more representative approaches. For example, in aquatic risk assessment, tier 1 risk assessment is based on single-species laboratory studies, and maximum PEC (Predicted Environmental Concentrations) values. Higher tier risk assessments can be based on mesocosms studies or modified exposure tests, which are intended to mimic more realistic exposure. These tests, that may be conducted with standard test species, are conducted to cover several exposure patterns, according to the use of pesticides in aquatic compartments. Experimental tests are indispensable, but can not be conducted indefinitely. TK/TD models, based on experimental data can be used to model biological reponses to these different exposure conditions. TK/TD models could become a very usefull tool for risk assessors in the next future. However, there is a need for agreed tools at EU level, which could be used by ecotoxicologists. A tool box, developped within academics, industry and regulators could be developped and widely used in a harmonised way for active substance monographs and/or for registration of plant protection products.

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Applying criteria for model evaluation to TKTD models
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Environmental risk assessment (ERA) relies on the comparison of results from an exposure and effects assessment. Estimation of exposure concentrations is based on mechanistic fate models, but for the effects side, the focus lies on toxicity testing and descriptive statistical treatment of data. Mechanistic effect models are gaining increasing interest in a regulatory context, and it is important that their 'quality' is established. Recent discussions on model evaluation and good modelling practice focus heavily on population and community modelling. However, effects models also exist at the individual level, generally falling into the category of toxicokinetic-toxicodynamic (TKTD) models. In contrast to the higher-level models, TKTD models are almost always completely parameterised by fitting them to a data set. In fact, one of their explicit aims is to replace the descriptive hypothesis testing and dose-response fitting as data-analysis tools. Furthermore, the development of these models generally does not fit nicely into an orderly modelling cycle; development of TKTD models is more like a complex interconnected web. These aspects have consequences for the application of frameworks for model evaluation to TKTD models. For example, formal sensitivity analysis becomes rather meaningless when all model parameters are fitted to a data set. And how do you validate a model that you fit to the data? I will illustrate these issues with the General Unified Threshold model of Survival (GUTS), working with a case study on propiconazole in *Gammarus pulex*, and provide recommendations for the evaluation of TKTD models.

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Using GUTS modelling in chronic effect assessment of a plant protection product on Americamysis bahia
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Effect assessments based on laboratory toxicity tests performed under constant exposure conditions may overestimate potential risks if the expected exposure profiles are characterised by a few short peaks. One option to evaluate effects from peak exposure is the use of a toxicokinetic-toxicodynamic model approach. In this modelling study, we use the General Unified Threshold model for Survival (GUTS) to analyse acute toxicity of an herbicide to the marine invertebrate *Americamysis bahia*, and to extrapolate lethal effects under long-term exposure from acute toxicity data. GUTS was calibrated using data from two independent acute tests. Model parameters and confidence limits were estimated based on the dose metric of the scaled internal concentration and the toxicodynamic assumptions of stochastic death (SD) and individual tolerance (IT), as formulated in the GUTS framework. Model verification indicated that GUTS, calibrated to the acute toxicity data described survival over time well, with acceptable confidence limits for the parameter variations. The parameterized GUTS was used to make predictions for *A. bahia* survival in two independent chronic tests. Long-term toxicity was better predicted with the IT assumption compared to SD. Nevertheless, both assumptions were used to justify the applicability of the TWA approach and to assess the chronic risk of short term exposure. Model predictions show that the reciprocity principle could be evidenced with the IT assumption but not with SD. Therefore, the TWA approach should not be applied in the chronic effect assessment of the herbicide on *A. bahia*. A peak exposure assessment using different criteria was conducted as a higher tier approach to link short term exposure to chronic effects. Mainly, ecotoxicological independence of peaks could only be demonstrated with the SD approach, so the link between exposure and effects should always be conducted on complete exposure patterns. Analysis of effect threshold mortality (EC_{10}) for increasing peak exposure durations showed an earlier onset of effects with the IT

assumption compared to SD. In conclusion, single peaks above the RAC could be assessed using a no relevant effect threshold, which represents a worst-case approach compared to the use of a NOEC. For multiple peaks, the peak duration above the RAC can be summed up and compared with the no relevant effect threshold.

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Linking the Adverse Outcome Pathway to Dynamic Energy Budgets: A conceptual model and two case studies
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Ecological Risk Assessment (ERA) quantifies the likelihood of undesirable impacts of stressors, primarily at high levels of biological organization: populations to ecosystems. Data come primarily from tests on individual organisms or from suborganismal studies, indicating a disconnect between primary data and societal concerns. We know how to relate individual responses to population dynamics using individual-based models, and there are emerging ideas on how to make connections to ecosystem services. By contrast, there is no established methodology to connect with suborganismal dynamics, in spite of progress in identifying Adverse Outcome Pathways (AOPs) that link molecular initiating events to ecologically relevant key events. We are co-PIs of a working group at the National Center for Mathematical and Biological Synthesis exploring the feasibility of using dynamic energy budget (DEB) models of individual organisms as a "pivot" connecting suborganismal processes to higher level ecological processes. AOP models quantify explicit molecular, cellular or organ-level processes, but do not offer a route to an integrated characterization of growth, reproduction, and survival. DEB models do describe these processes, but use abstract variables with undetermined connections to suborganismal biology. We propose linking DEB and quantitative AOP models by interpreting AOP *key events* as measures of damage-inducing processes in a DEB model. Work is in progress on two case studies based on *Daphnia* and on rainbow trout. For rainbow trout, we focused on endocrine disruption for which there are quantitative AOPs that integrate molecular, cellular and organ level responses to predict effects on precursors of reproduction. Connecting with a DEB representation required modifying the "standard" DEB model to include feedbacks that characterize the integrated effects of hormonal control mechanisms, but *mechanistic connection* was achieved. With *Daphnia*, there is little organ level data, so we seek *correlative* connections with transcriptomic data. *Daphnia* were exposed to a gradient of food rations and contaminant concentrations over time with measurements of gene expression and contaminant body burdens along with routine measurements of size, survival and reproduction. Gene expression data (interpreted as key events), summarized using Principle Component Analyses appear to exhibit some molecular responses that may correlate with parameters controlling relevant DEB fluxes.

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An individual-based model for the three-spined stickleback to assess population-level effects of EDC-induced disruption of breeding behaviours
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Population modelling has the potential to be employed to extrapolate from individual- to population- level effects in the environmental risk assessment (ERA) of chemicals. Population models that incorporate ecological processes such as density dependent competition, individual variability and aspects of behaviour would add further realism to risk assessment. This is further evidenced by the European Commission's recent inclusion of population modelling as a potential tool for identifying the population relevance of effects within the draft criteria for the identification of endocrine disrupting properties of chemicals. Here, we present an individual-based model (IBM) for the three-spined stickleback with the purpose to simulate realistic scenarios relevant for assessing the effects from exposure to EDCs. The three spined stickleback is widespread geographically, and potentially sensitive to a number of potential effects given its complex breeding strategy, low fecundity and the provision of high level of parental care. This IBM has been structured using a series of sub-models, based on empirical data obtained from published literature. Density dependent reproduction, growth, and mortality along with individual breeding behaviours are key parameters within the model. In the three-spined stickleback (*Gasterosteus aculeatus*) it has been shown that nest building, courtship displays and parental care, may be disrupted by exposure to certain endocrine disrupting chemicals (EDCs), possibly affecting population recruitment. We describe the development, validation and sensitivity of the stickleback IBM and display its potential for application in the ERA of EDCs using EDC-induced disruption of breeding behaviours as an example endpoint.

Organic micropollutants in the environment: analytical challenges and engineering innovations (I)

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Using polyethylene passive samplers of varying thickness to measure dissolved organophosphate ester flame retardants in the North Atlantic Ocean
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Organophosphate esters (OPEs) have been used as flame retardants and plasticizers in a wide variety of consumer products. Previously, OPEs were thought to degrade readily in the environment, and so they have increasingly been used as replacements for other persistent halogenated flame retardants. However, recent research has shown that OPEs can persistent and are capable of long-range transport. In this study, extracts from polyethylene passive samplers (PEs) of various thicknesses that underwent year-long deployments in the eastern and western Fram Strait were retrospectively analyzed for three chlorinated and nine alkyl/aryl OPEs. PEs with thicknesses of 50 µm, 800 µm, and 1600 µm were deployed from July 2012 to July 2013 and from June 2014 to July 2015 at depths ranging from 248 to 2535 m. OPEs were most frequently detected in extracts from 50-µm PEs, which were deployed during the 2014-2015 sampling campaign. These thin PEs were assumed to have reached equilibrium during year-long deployments. Two chlorinated OPEs (tris(1-chloro-2-propyl)phosphate (TCIPP) and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP)) made up >70% of total OPEs in all samples. Dissolved concentrations of TCIPP were estimated to range from below detection limits (< DL) to 465 pg/L, while for TDCIPP concentrations ranged from 8 to 38 pg/L. Among the non-chlorinated OPEs, tri-n-butyl phosphate (TnBP), triphenyl phosphate (TPHP), 2-ethylhexyldiphenyl phosphate (EHDPP), and tris(2-ethylhexyl)phosphate (TEHP) were frequently detected, with TnBP at the greatest concentrations, ranging from

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Toward the prediction of sampling rates: Sorption and permeation properties of membrane filters used for aquatic passive samplers
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Passive, integrative, in-situ aquatic samplers such as the polar organic chemical integrative sampler (POCIS) and Chemcatcher have received increasing attention in environmental research as they allow the measurement of more ecotoxicologically relevant, time-integrated concentrations. To perform passive sampling in the field, the apparent uptake rate or the sampling rate (Rs) needs to be known. Rs is specific to the sampler, the chemical, and partially to environmental conditions. Accepted models to predict Rs for many chemicals, however, do not exist so far, as Rs does not appear to be a simple function of one chemical property. As a first step toward a mechanistic model for the Rs prediction, this work characterizes two membrane filters used for aquatic passive samplers, polyethersulfone (PES) and polytetrafluoroethylene (PTFE) filters, with respect to their sorption and permeation properties. Through the sorption and permeation experiments conducted, we found: (1) PES filter exhibited rather strong sorption, with PES-water partition coefficients well above Kow. (2) In contrast, PTFE did not show any significant sorption for the chemicals used in this study. (3) Permeation through membrane filters of non-sorptive tracers can be fully described with diffusion in aqueous boundary layers (ABL) and in membrane pores. (4) In case the sorption by membrane filter is significant, local equilibrium sorption in membrane pores can be assumed to describe the permeation through the filter. Based on these results, a PTFE membrane filter is recommended to use as a general-use filter material which is expected to avoid a possible lag time and enable a relatively quick response to the fluctuating concentration in water. A further study is suggested to elucidate kinetic sorption behavior by sorbents inside the passive samplers, which appears to represent a significant fraction of the total permeation resistance in the sampler.\n\n

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Passive samling as a screening tool for determining the influence of hospitals on emissions of pharmaceuticals
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The occurence of pharmaceuticals in the environment is an emerging concern, and with both the ageing anf teh growth of the global population, overall use and consequently emissions to the aquatic environment will most probably increase. In the forthcoming decades ther will also be a shift in use from developed to so-called pharmemerging countries. In order to take adequate and effective measures to reduce environmental concentrations, insight in the most dominant sources is crucial. In this study the influence of two large hospitals on the overall load of pharmaceuticals on the Sewage Treatment Plant (STP) in two major cities inteh Netherland was determined. Next to this, the removal rate of teh different pharmaceuticals on teh STP was determined. For this we deployed passive samplers (speedisks) on different locations int the sewage sytem for a number of days.After deplyment, the samplers were extracted and analysed on in total 80 pharmaceuticals, divided over different groups, like antibiotics, contrast media, cytostatics and analgesics. Based on earlier results, we were able to calculate

aqueous concentrations from the concentrations on teh samplers, and consequently loads, using the flow rates of teh different locations. The chemical analyses showed that passive sampling increases teh number of compounds detected in the sewage, compared to grab sampling. As expected, highest concentrations were found in the sewage of the hospitals. Within the groups of compounds, large differences exist between the hospitals, probaly caused by the different apllication patterns within the hospitals. Based on the load calculations, the hospitals appeared to be the main source, but for most compounds, other sources like houosehoulds and nursing homes appeard to be most important. Analyses of the influent and effluent concentrations of the STP showed that the removal efficiency was highly variable, even within groups of compounds. Thes efindings show that defining measures in order to reduce emissions of pharmaceuticals nee sto be custom fit, based on purpose fit monitoring. Passive sampling is an effective tool for this.

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Environmental forensics in seawater by coupling a divinylbenzene passive sampling device and high resolution mass spectrometry for the screening of organic micropollutants

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Environmental analysis encounters several difficulties such as fluctuating concentrations (due to variation in space and time), complex mixtures of micropollutants and different transformation products of the same emerging substances (due to environmental conditions). Combining passive sampling with high-end ultra-high-performance liquid chromatograpy and high resolution mass spectrometry (UHPLC-HRMS) will improve environmental measurements. Indeed, passive samplers accumulate compounds during exposure, improving the detection of organic micropollutants. The aim of this study was to sequester polar to non-polar emerging organic micropollutants in the marine environment (harbour of Zeebrugge, harbour of Oostende and open sea; all located in the Belgian Part of the North Sea) by using a divinylbenzene (DVB) passive sampler of which the extracts are analysed by two in-house validated UHPLC-HMRS methods. Comparing the results of these two methods eliminates the analytical preconceptions of the organic micropollutants' physico-chemical preferences, caused by sample treatment and chromatographic and ionization conditions. The raw data of the HRMS analyses were processed by three different approaches, i.e. target, suspect and non-target screening. The target screening was based on the preselection of 145 organic micropollutants, including pharmaceuticals, personal care products, pesticides, natural and synthetic steroids. In total, 60 out of these 145 target compounds were detected and quantified in the passive sampler extracts. The suspect screening, using a database compiled through scientific literature data, enabled the identification of 380 micropollutants, including the same therapeutic classes as the target analytes. The suspect screening approach revealed the occurrence of 124 out of the 380 suspected emerging compounds in the collected samples. The non-target screening combines multi-variate analysis and online databases for the identification of unknown micropollutants. Non-target screening demonstrated additonally that 14 emerging organic pollutants co-occur at each sampling loaction. Overall, it can be concluded the DVB passive sampler coupled to UHPLC-HRMS methods has demonstrated promising results for target, suspect and non-target screening, contributing to a specific molecular fingerprint of the different sites sampled in the marine environment.

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In situ equilibrium passive sampling of hydrophobic organic hydrocarbons in coastal marine sediments Comparing in situ versus ex situ sampling

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The study provides the first comprehensive dataset on in situ measurements of freely dissolved concentrations and chemical activities of PAHs and PCBs in coastal sediments of the North Sea and the Baltic Sea. During a research cruise in summer 2015, 50 in situ samplers were deployed in the coastal area of Germany. After three months 37 of the samplers were recovered. SPME fibers with different coating thickness (10 μm and 40 μm) were collected and analyzed in the laboratory. During both cruises surface sediment samples were simultaneously collected at the same sampling stations. For in situ sampling an equilibrium passive sampling device was used that facilitates the in situ measurement of hydrophobic organic chemicals (PCBs and PAHs) bioavailability in sediments in terms of freely dissolved concentrations. The sampler is applicable in a multitude of aquatic environments, especially where currents are low and sediments are muddy and well-mixed e.g. by bioturbation. Examples for such environments are mud flats, harbor basins, river banks and lakes. The field sampler allows PDMS fibers and hollow fibers to be immersed and equilibrated in situ, whereas an automated liner exchanger (ALEX) facilitates the quantitative transfer of analytes to the GC without the use of extraction solvents. Additionally, ex situ SPME experiments of these sediments were performed in the laboratory with the same analytical method. Experimental parameters like temperature and exposure time were adjusted as to meet the conditions of the corresponding experiment with the in situ sampling device. Finally, the results of the in situ sampling were compared with ex situ laboratory experiments. On the basis of the experimental findings, advantages and disadvantages of ex situ versus in situ measurements are discussed. From the in situ and ex situ experiments we examined (i) spatially resolved freely dissolved PAH concentrations (C_{free}); (ii) baseline toxicity potential on the basis of chemical activities (a); (iii) site specific mixture compositions and (iv) site specific distribution ratios.

Advances in Soil Ecotoxicological Risk Assessment of Chemical Stressors (II)

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Prioritisation and screening risk assessment of contaminants in sewage sludge for application to land

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The re-use of sewage-derived biosolids on land is a balance between sustainable use of a potentially valuable resource and concern over possible degradation of the environment. Wca has undertaken an assessment of known chemicals of concern and emerging contaminats in biosolids arising from sewage treatment. An evidence-based, precautionary risk screening assessment was developed to prioritise chemicals for routine measurement in biosolids that may be applied to agricultural land. Changing social and cultural norms across the developed world over the last 20 years have raised researchers interest in the determination of the presence of new groups of chemicals in biosolids. These groups of chemicals include personal care products, industrial organic chemicals, biocides, human and veterinary medicines, road-wash chemicals, plasticisers and flame retardants. The strategy used here for contaminant prioritisation is based upon: occurrence as indicated by literature search and review of regulatory surveys of biosolids (identification of chemicals of concern and compilation of representative concentrations); environmental fate and behaviour characteristics to assess accumulation in sewage sludge & persistence in soil; & preliminary ecological risk assessment for terrestrial environment and ranking of toxicological potency The literature survey provided a list of 158 organic chemicals detected in biosolids. The initial screening based on fate and behaviour characteristics indicated 24 organic compounds to be taken forward for risk assessment in addition to trace elements routinely measured in biosolids. Risk characterisation for the terrestrial environment based on application of biosolids to land at different rates and over variable time periods showed an unacceptable risk for copper, zinc, dieldrin, triclosan and HHCB (Galaxolide) under all scenarios with other chemicals only presenting a potential risk at the highest application rates. The results of this screening and prioritisation exercise highlights the chemicals likely to be of most concern in biosolids applied to land and can be used to guide the selection of priority contaminants for future monitoring. These findings should be confirmed by analysis of contaminants in biosolids and possibly also in agricultural soils to which sewage-derived biosolids are applied. *Acknowledgement* - The authors thank New South Wales Environment Protection Authority for funding this work

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Ecotoxicity of Cu Substances in Soil - Assessment of Moiety-based Approach

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The use of an inorganic metal moiety approach has been identified as a risk assessment tool under the Government of Canada's Chemicals Management Plan. Given the volume of priority chemicals requiring evaluation under the CMP program, a metal moiety-based approach has been recommended to stream-line the risk assessment process for inorganic substances. However, there is some uncertainty surrounding the metal moiety approach as it relates to toxic responses to organisms or the microbial community in soil systems. As a result, Cu-containing substances were assessed using a grouping-based approach: an organometal (copper dimethyldithiocarbamate); a water-soluble inorganic salt ($CuSO_4$); a water-insoluble inorganic (CuO); a water-soluble organic metal salt (copper D-gluconate); and a water-insoluble organic metal salt (copper gluconate). Soil ecotoxicity effects were assessed in an amended sandy soil using a combination of single-species plant (*Elymus lanceolatus* and *Trifolium pratense*) and invertebrate (*Folsomia candida* and *Eisenia andrei*) tests, and a suite of soil microbial tests. Soil samples were analyzed so that effective concentrations could be derived on a nominal basis, a total Cu basis, a total parent compound basis for organic copper substances, and well as Cu^{2+} activity. Toxicity varied by substance, test species and microbial test. The use of ion activity was critical to demonstrating the availability and influence of the copper ion on toxicity across test substances. The use of a test battery encompassing both single-species and microbial endpoints provides added strength to the ecological risk assessment process in that in some instances, microbial endpoints were as sensitive as the most sensitive test species (*E. andrei*). Our study data provides a case example that could help validate guidance on risk assessment approaches for complex metal substances and brings into question the appropriateness of the metal moiety approach for the estimation of risk posed by certain organic metal salts and organometals.

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Do microbes matter: metal mixture inhibition of microbial processes influence ecosystem services.

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Soil microbes, invertebrates and plants play an essential role in ecosystem services. The inclusion of microbial funtions function into environmental regulations is at early-stage. A key step in the adoption of microbial endpoints is unravelling the importance of microbe's indirect roles in supporting ecosystems. These roles can be termed Ecosystem Services and are are defined as the benefits people obtain from ecosystems. These include provisioning services such as food and water; regulating services such as flood and disease control; cultural services such as spiritual, recreational, and cultural benefits; and supporting services, such as nutrient cycling, that maintain the conditions for lifeh. This study will focus mainly on the supportive, provisional and regulatory services that involve microorganisms and how metal contamination alters the provision of these ecosystem services. This study seeks to explore the relationship between metal mixture toxicity on microbial communities and ecosystem services. Sterile and non-sterile soils (n=41) were dosed 2500 mg/kg of a mixture contaning lead, copper,zinc, cobalt and nickel. This concentration was previously determined from preliminary to inhibit approximately 50% of microbial activity, and compared to soils that were not dosed with the mixture. Northern wheatgrass was then grown in each of these soils for 35 days, and five ecosystem services were assessed: (i) Forage quality, (ii) Xenobiotic degradation, (iii) Climate regulation, (iv) Organic matter decomposition and (v) nutrient cycling. Forage quality was determined through protein content of the forage grass, xenobiotic degradation via glyphosate degradation, climate regulation by methane, carbon dioxide and nitrous oxide emission rates, organic matter degradation by beta glucosidase activity, and nutrient cycling via nitrification and phosphatase enzyme activity assessments. The metal mixture exerted much greater toxicity towards autotrophic nitrification in sterile soils compared to non-sterile controls. In contrast, sterility had little effect on metal inhibition of beta-glucosidase or phosphatase treatments. Other ecosystem service assessments are currently in progress and will be completed in the new year. Metal mixture inhibition varied greatly amongst endpoints and between soils. Future work will be assessing oribatid mite inhibition in these same soils to link microbial and ecosystem service endpoints to a soil toxicity test as well.

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Juvenile soil invertebrate avoidance to soil contaminants

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Avoidance tests have been developed in recent years to assess the avoidance behaviour of soil organisms when exposed to contaminated soils as they are quick and inexpensive. These tests are useful for ecotoxicology risk assessments as avoidance of contaminated soils can reduce the ecosystem services provided by soil organisms and can impact the energy budget of the organisms. Existing literature on avoidance of soil invertebrates utilize adult individuals and lack juvenile responses. Avoidance of juvenile organisms to contaminated soil may be a more or less sensitive endpoint. The sensitivity of juveniles to contaminated soils influences the habitat range of populations and influences the soils ability to provide ecosystem services on contaminated sites. The objectives of this study were to determine the EC25 for juvenile avoidance to three contaminants and compare to published literature for adults results. Three contaminants, phenanthrene, dimethoate, and copper sulfate, were chosen to represent different classes of contaminants. Four species of soil invertebrates, *Oppia nitens*, *Folsomia candida*, *Hypoaspis aculeifer*, *Eisenia fetida*, and *Enchytraeus crypticus* were tested with each of the contaminants. Artificial soil prepared to the guidelines of the OECD was used for each experiment. Net response to the contaminant was calculated to determine if there was an avoidance or an attraction response. The EC25 values for juvenile avoidance were calculated using the Weibull function. Preliminary data for copper suggests that juvenile *Eisenia fetida* shows avoidance, but at higher concentrations than adult avoidance. *Folsomia candida* showed a lack of preference for either clean or contaminated soil up to concentrations of 10000 mg/kg which is well above the threshold for reproductive effects in collembolan species.

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Effect of chemical and biofumigants on ecotoxicology of earthworms (Eisenia andrei) and soil microbial communities

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Biofumigation is considered a good alternative to chemical fumigation because it can control crop pathogens and diseases with lower health and environmental risks. Glucosinolates are volatile compounds found in most *Brassica* species and when hydrolysed, forms a range of natural toxins that act as biofumigants. Efficacy of both chemical and biofumigants on target species have been well documented, however, very little information is available on its effects on non-target species. Negative effects on beneficial soil organisms can have serious negative impacts on soil quality especially when essential ecosystem functions are affected. Earthworms and soil microbial communities are important indicators of soil quality due to their prominent role in the productivity of soils. Three biofumigants, broccoli, mustard and oilseed radish, and two chemical fumigants, metham sodium and cadusafos, were investigated for possible effects on non-target and essential soil organisms. The ecotoxicity of fumigants was evaluated utilising a standardised earthworm ecotoxicity test determining survival, growth and reproduction. The genotoxicity was assessed with the comet assay to investigate the extent of cellular DNA damage of earthworm colomic cells. Changes in the soil microbial community function and structure were evaluated by means of Biolog™ Ecoplates and phospholipid fatty acid (PLFA) analyses respectively. Broccoli reduced earthworm reproduction while mustard induced more DNA strand breaks in earthworm cells compared to the control. Effects exerted on the microbial community were most pronounced within the first 14 days after application. Mustard showed a pronounced effect after 28 days but had no lasting effect on the functional diversity.The chemical fumigants had a marked negative impact on the ecotoxicity and the genotoxicity of the earthworms. Both chemicals had an inhibitory effect on the microbial growth in terms of the viable biomass but no lasting effects were observed in the community structure. Metham sodium treatment showed limited effects on the functionality of the microbial community whereas cadusafos had a noticeably different effect. The varying effects of one treatment on different organisms, illustrates the importance of using different bioindicators to get a better understanding of the overall effects on the soil ecosystem.

Challenges and best practice in monitoring of micro- and nano-plastic abundance and environmental distribution (II)

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Current status of plastics in European freshwater environments - results of a European survey

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There is less knowledge on plastics in freshwater environments compared to the plastic pollution in the seas. Currently, it is assumed that the occurrence in limnic systems has effects in ecosystems comparable to that identified for the marine environment. In addition, riverine inputs are regarded as a significant source of plastics in the oceans. In 2016, the German Environment Agency (UBA) and the German Federal Institute of Hydrology (BfG) organised a “European Conference on Plastics in Freshwater Environments” on behalf of the German Federal Environment Ministry. In preparation of the conference, a survey was conducted on the current state of knowledge and activities in the various European countries with regards to plastics and freshwater. The issues addressed included monitoring and effect studies of plastics in freshwaters, riverine loads and inputs into the seas, main sources and pathways as well as risk perception and management options. A questionnaire was informally sent to the representatives of the European countries responsible for the implementation of the water Framework Directive. Twenty-eight EU Member States and six other European countries were addressed. Fourteen of these countries participated in the survey. Survey responses and references were supplemented with a respective literature research for an issue paper. The results of the survey showed that monitoring studies cover only part of the European freshwaters. Currently, data on temporal trends are missing. Comprehensive monitoring is hampered since methods of sampling, sample processing and analytical identification have not been harmonised so far. Little is known about the fate of plastic materials in freshwaters. Only roughly estimated data are available on sources, riverine loads and inputs into the seas. Research on effects of MP and mesoplastics in freshwaters is just at the beginning. Sor far, potential effects of additives and adsorbed pollutions have not been thoroughly assessed. The survey showed that risk perception by media, social networks and NGOs is on average in most participating countries while three countries showed higher rankings. Reduction measures are mostly discussed by NGOs and the media, followed by public and regulatory agencies. The majority of the surveyed countries are planning or have already implemented measures to reduce the plastic inputs into freshwaters.

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Monitoring of microplastics in blue mussels (Mytilus edulis) from the French Atlantic coast (Pays de la Loire, France)

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According to the last ten years of research, MPs have been detected in all natural habitats from pole to pole and in all environmental compartments: marine and fresh waters, sand, sediments and aquatic organisms. Monitoring the presence of MPs in marine organisms is currently of high importance as some of these organisms such as mussels and oysters are consumed by human and may constitute the main vector of human contamination. Hence, the aim of this study was to evaluate the contamination of blue mussels (*Mytilus edulis*) from the French Atlantic coast (Pays de la Loire, France) which is a bioindicator, filter-feeder species with an important socio-economic weight. Blue mussels studied in this work were sampled in two sites of the French Atlantic coast, in the Pays de la Loire region at two different seasons: autumn 2015 and spring 2016. Cultured and wild organisms were collected to be compared. A total of 120 mussels were analyzed. First, a fast protocol for isolation and identification of MPs without visual sorting was set up and validated using spiked samples. Pools of 3 mussels were subjected to digestion, sedimentation and filtration. MPs were then detected and identified directly on the membrane filters using μFTIR spectroscopy in reflection mode. More than 60 MPs of seven different chemical natures were found and identified in the sampled mussels, PP and PE representing more than 80% of the identified MPs. Their size ranged from 20 to 400 μm with 89% of the particles with size inferior of 100 μm. The concentration of MPs was evaluated as an average of 1.6 MPs per pool of 3 mussels and 0.2 MPs per gram of soft tissue. Besides, 90% of the cultivated mussel pools were contaminated *vs* only 60% for the wild mussel pools. Significant differences in the quantities according to the mussel type (wild / cultivated) and the sampling site were observed. One explanation could be that cultivated mussels were exposed to more plastic objects (like nets) than wild mussels. This work provides the first dataset on the level of contamination of bivalves from the French Atlantic Coast.

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Release of Synthetic Microplastic Plastic Fibres From Domestic Washing Machines
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Washing clothes made from synthetic materials has been identified as a potentially important source of microscopic fibres to the environment. In this study, common synthetic fabric types (polyester, polyester-cotton blend and acrylic) were laundered, and the fibre release examined. These fabrics were laundered under various conditions of temperature, detergent, and conditioner. Fibres from waste effluent were examined and mass, fibre size and abundance compared between treatments. Average fibre size ranged between 5.0–7.8mm in length, and 11.9–17.7μm diameter. Polyester-cotton consistently shed significantly fewer fibres than either polyester or acrylic. However, fibre release varied according to wash treatment with various complex interactions. It is estimated that over 7000 fibres could be released from an average 6kg wash load of acrylic fabric. Fibres have been reported in effluent from sewage treatment plants; our data indicate fibres released by washing of clothing could be an important source of microplastics to aquatic habitats.

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Microplastic in water, sediment, invertebrates and fish living in urban stormwater wet detention ponds
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Microplastic as environmental pollutant is of worldwide concern. Scientists and authorities typically define microplastic particles as being between 1 μm and 5 mm in size. They can originate from products, where they are added intentionally to entail a scrubbing or abrasive effect, or be derived from fragmentation of bigger items of plastic litter that are tossed and detained in the environment. Scandinavian studies have concluded that most of the microplastic that enters the sewer systems is effectively removed from the water stream and accumulated in the sewage sludge. However, some of the sources of microplastic entail emissions that are diverted to the stormwater runoff, which many places in Denmark are separated from the sewage and treated to a lesser degree before discharge to the surface waters. A common technique for stormwater management is wet detention ponds, where the runoff is detained and pollutants retained by processes such as sedimentation, sorption, and biological degradation. Even though, the ponds are technical treatment facilities, they accommodate wildlife e.g. invertebrates and fish, which finds them attractive as habitats for feeding and breeding. This entails that the organisms living in the ponds are exposed to the microplastic that are carried with the incoming stormwater runoff. The objective of the study was to provide information about the prevalence of microplastic in wet detention ponds – and determine whether the plastic are present in the water, in the sediment, or even in the fauna existing in the ponds. Furthermore, an important part of the study was to develop and validate different methods for extracting microplastic from a variety of matrices e.g. pond water, sediment and fauna. Several different methods were compared – where all samples were added microplastic beads of polystyrene to allow determination of recovery through the whole analysis process. The microplastic is determined by FT-IR (Fourier Transform Infrared spectroscopy) imaging applying a Focal Plane Array. Hereby, the pixel resolution allows microplastic determination down to approx. 10 μm particle sizes. This study contributes with knowledge not only on the fate of microplastic particles in freshwater systems affected by urban runoff, but also in the aquatic environment in general. Furthermore, the study presents methods for extracting microplastic from pond water, sediment and fauna to make the sample suitable for quantification for FT-IR imaging.

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Poster spotlight: WE131, WE132, WE139

Integrated approaches for linking chemical contamination with biological effects

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Chemical and toxicological profiling of large rivers using mobile passive sampling and comparison to large volume active sampling
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Risks associated with complex mixtures of chemicals in freshwaters are difficult to predict. Efficient sampling methods and sensitive effect based tools can significantly improve the risk assessment. Novel mobile "enhanced" passive sampling system was applied within the Joint Danube Survey 3 in 2013. The partitioning samplers for non-polar compounds, i.e. silicone rubber sheets, and an adsorption sampler for polar compounds based on Empore SPE disks integrated water pollutants in time and space over 8 river stretches. Spot sampling of high water volumes was conducted by large volume solid phase extraction at 22 sites along the river. Up to 500 L of water were passed through extraction system containing neutral sorbent Chromabond HR-X, anionic exchanger Chromabond HR-XAW, and cationic exchanger Chromabond HR-XCW. Extracts of samples were subjected to analysis of organic pollutants and to toxicological profiling. Spatial profiles of a broad range of organic pollutants and studied toxicological endpoints were identified. In many cases the integrative character of passive sampling allowed measurement of compounds at pg L^{-1} levels. Besides oxidative stress response, toxic equivalents were detected in a similar range for active and passive sampling. The passive sampling reflected the physical-chemical characteristics of the compounds driving individual effects. While the PXR-mediated and estrogenic effect potentials were detected namely in Empore samplers, thus were elicited mainly by polar chemicals, AhR-mediated effect potential was primarily detected in silicone rubber samples, which reflects significant contribution of less polar compounds. The bioanalytical equivalent concentration approach was used to characterize the contribution of the detected chemicals to observed toxic potentials and identify the main toxicity drivers. The fraction of biological effects explained by the detected chemicals in samples from passive sampling of water from the Danube river differed depending on the studied effect and the type of sampler. Our study demonstrates the utility of the integrated approach using toxicological profiling to characterize pollutant mixtures along with chemical analysis in monitoring of fresh water quality and good applicability of passive sampling and large volume solid phase extraction in this assessment. This research was supported by EU FP7 Project SOLUTIONS (no. 603437).

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Effect-directed analysis (EDA) of Danube River water samples receiving untreated municipal wastewater from Novi Sad, Serbia.
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Abstract: Untreated wastewater emitted to the River Danube may deteriorate the

surface water quality consequently having adverse effects on human health and on aquatic life including endocrine disruption and oxidative stress. In this study, effect-directed analysis (EDA) has been applied to identify chemicals that may impact on different hormonal pathways and cause oxidative stress. Water samples from Danube River were extracted on-site using large volume solid phase extraction (LVSPE) device and fractionated using reversed phase-high performance liquid chromatography (RP-HPLC) in order to reduce sample complexity. GeneBLAzer bioassays and AREc32 bioassay were used for the detection of agonistic and antagonistic hormonal activity for four nuclear hormone receptors (estrogen (ERα), androgen (AR), progestagen (PR) & glucocorticoid (GR)) and of oxidative stress respectively. Chemical Analysis was performed by using LC-HRMS/MS and LC-MS/MS. Biological analysis identified several active agonistic fractions on nuclear hormone receptor endpoints but none of the fraction showed any antagonistic effect. Oxidative stress could be detected only in the parent sample and the recombined mixture but not in the fractions indicating a mixture effect rather than outstanding individual toxicants causing this effect. Chemical Analysis of the active fractions revealed natural steroidal hormones responsible for activity in ERα and AR, while chemical analysis of PR and GR is ongoing. Mass balances on the basis of individual chemicals and testing chemical mixtures with the same relative concentrations as they were found in chemical analysis confirmed that most of the activity in environmental fractions was caused by the detected chemicals. Endocrine disruption potential of water samples was explained very well by detected chemicals. Natural steroidal hormones including female and male reproductive hormones were found to be playing a major role for biological effects in terms of endocrine disruption. Oxidative stress response was found to be a mixture effect that does not allow the identification of individual chemicals causing this effect. Monitoring of contamination causing oxidative stress has to fully rely of effect-based measurements.

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A novel fractionation approach using four columns in parallel for effect-directed analysis of antiandrogenic compounds in a river water extract

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Endocrine disrupting compounds in the environment can cause reproductive disorders in human and wildlife. Increasing evidence underline the impact not only of the already extensively investigated anti-/estrogens but also anti-/androgens on this kind of disorder. For example the occurrence of feminized fish in river water has been associated not only with estrogenic but also antiandrogenic compounds. EDA is a powerful tool for the identification of unknown toxicants. The fractionation procedure essentially influences the success of each EDA. It is classically performed in a sequence of fractionation, biotesting and selection of active fractions for the next fractionation step until the complexity has been reduced to a small number of compounds, for which structure elucidation is carried out. In this study a novel, time efficient fractionation approach using 4 orthogonal columns in parallel is introduced. Chemical and toxicological analysis is conducted on fractions from the different separation systems. The candidate peaks common in bioactive fractions are subjected to toxicant identification. For the selection of orthogonal LC stationary phases the retention times of 39 (anti)androgens were determined on 17 different stationary reversed-phases. The grade of orthogonality was evaluated using different statistical approaches. Four columns were selected: aminopropyl-, octadecyl-, pyrenyl ethyl and a pentafluorophenyl phase. A novel, downscaled antiAR-CALUX assay using low-volume exposure and chemical dilution procedures was applied for biotest of fractions. Four fractions, one of each column, exhibited antiandrogenic activity in the downscaled antiAR-CALUX assay. In average 3300 peaks were detected in each of those fractions using HR-MS/MS. Solely 24 of those peaks were found in common in all 4 active fractions. This corresponds to a 99.3% reduction of MS-data complexity for further analysis, clearly underlining the great potential of the novel fractionation approach. Three compounds, yet not known as antiandrogenic active, were identified: 4-Methyl-7-diethylaminocoumarin (C47), 4-Methyl-7-ethylaminocoumarin (C47M1) and 7-Amino-4-methylcoumarin (C47M2). The antiandrogenic activity of the identified compounds was further evaluated in a higher trophic level using the *spiggin-gfp* Medaka. The high antiandrogenic potency and thus environmental relevance of C47 and C47M1 was confirmed.

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Mutagenicity in Surface Waters: Is it complex mixtures or individual chemicals?

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Mutagenicity is a major concern for drinking water production and frequently detected in surface waters. Several studies point out the contribution of aromatic amines to the observed mutagenicity of rivers but so far no individual mutagen was identified. Therefore, the aim of this work was to examine the mutagenicity of mutagenic surface water samples focusing on aromatic amines in two case studies in the rivers Rhine and Mulde and to understand whether individual pre-dominant mutagens or mixture effects are the drivers of mutagenicity in these rivers. A full EDA study was applied to the sample of River Rhine and a virtual EDA study in River Mulde was conducted focusing only on aromatic amines. *River Rhine:* A sample collected with an on-site LVSPE machine was fractionated and the mutagenicity of the fractions was assessed with the strain TA98 and YG1024 both with metabolic activation by S9 and fractions with an increased activity with the YG1024 were further analyzed. *River Mulde:* Six samples were collected with the passive sampler Blue Rayon (BR) in six consecutive weeks. The mutagenicity of the BR extracts was evaluated with TA98 strains with S9. The selected fractions of River Rhine and the samples of River Mulde were subjected to a previously developed derivatization method to label aromatic amines and analyzed with a Q-Exactive Plus (Thermo) instrument. The MS/MS of the analytes with confirmed derivatives were used to generate candidate lists with Metfrag 2.2. Hydrogen-deuterium exchange and pH dependent LC retention were applied for candidate selection. Fourteen fractions of River Rhine were found mutagenic with the strain YG1024 indicating that the mutagenicity is caused by a large number of mutagenic compounds. 21 compounds were identified in the active fractions from different compound classes including industrial aromatic amines: o-toluidine and 2,6-xylidine, alkaloids including the co-mutagen norharman and two of its isomers carboline and 5-carboline. Mixture tests revealed a strong synergism of the identified aromatic amines with the carboline isomers. In the River Mulde, among the 13 identified compounds of BR extracts 2,3- and 2,8-diaminophenazine were found to be the major drivers of the mutagenicity in River Mulde. These results indicate that aromatic amines are important mutagens in surface waters and play a major role as pre-dominant mutagens or key compounds in a mixture effect.

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Nationwide screening of herbicide risk to algae

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According to the European Water Framework Directive (WFD), chemical water quality is assessed by monitoring 45 priority compounds. This list is outdated however, as the selected compounds are not representative for present day contamination. Consequently, toxic effects observed in surface waters, can often not be attributed to compounds measured by the water authorities. Hence, there is an urgent need for a more effect based monitoring strategy that employs bioassays to identify environmental risk. An adequate selection of bioassays is crucial for this monitoring strategy, as identification of compounds causing the observed effects depends largely on this selection. Algal photosynthesis is a sensitive and well-studied process to identify the presence of hazardous herbicide concentrations in surface water. Therefore the aim of this study was to develop and apply an innovative algal photosynthesis bioassay to assess surface water herbicide risk to algae. To this purpose, *Pseudokirchneriella subcapitata* was exposed to surface water samples in 96-well plates. After 4.5 hours, effective photosystem II efficiency (φPSII) was determined using Pulse Amplitude Modulation fluorometry connected to a robot, resulting in a rapid high throughput bioassay. Yet, 54% of the test plates had to be excluded, mainly due to oversensitivity to the positive control (atrazine). Hence, constancy of algal sensitivity should be improved for future application of this bioassay. With the remaining valid bioassays, potential effects of surface water from 39 locations were assessed. Algal photosynthesis was affected by surface water from only one location. Chemical analysis of this single toxic sample revealed that three herbicides were present above the environmental quality standard (EQS) concentrations. The observed effect could largely be attributed to one of these herbicides, linuron, which occurred at 110 times the EQS concentration and which is not included in the WFD priority compounds list. In conclusion, applying the algal photosynthesis bioassay may avoid redundant chemical analyses, while simultaneously identifying the presence of hazardous compounds that would have been overlooked by routine chemical WFD monitoring. Key words: Algal bioassay, PAM fluorescence, Herbicides, Surface Water Toxicity

LCA for supporting policy and decision making

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Applying LCA in decision making- the need and the future perspective

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There is nowadays a need of including sustainable considerations in the policy and decision making. Sound decision making requires evidence-based support, i.e. decision analysis to help decision makers in identifying the best alternative based on the associated impacts. Decision analysis includes four steps: 1) structure decision problem; 2) assess possible impacts associated with alternatives; 3) determine stakeholder preferences and 4) evaluate alternatives. Decision analysis can be performed applying different tools, such as cost-benefit analysis (CBA), risk assessment, and life cycle assessment (LCA). LCA is a decision analysis tool that focuses on environmental impacts. One limit is that LCA is based on defined impact categories and therefore does not provide information for those impacts and consequences out of the LCA scope. However, the LCA framework closely follows the decision analysis scheme and has the potential to be integrated with other decision analysis tools to enhance their assessment of environmental impacts. To understand why LCA is needed in the policy decision context, we looked into the decision support for policy in several disciplines. Taking sustainable transport policy as an example, the traditional decision analysis tool for choosing the best alternative is CBA. CBA mainly analyses socio-economic impacts, such as travel time savings and costs, while only some environmental impacts are considered; i.e. the damage costs of greenhouse gas emissions, particulate matters, SOx, NOx and noise. Therefore, current transport policy making rarely reflect a full environmental profile of the suggested alternatives. Making decisions based on incomplete information may lead to sub-optimal solutions, especially where the environment is a major concern. There is a growing attention of conducting LCA in transport. Some identified environmental hotspots, such as consumer and household behavior, which may be the focus for future policies. Others assess the environmental impacts associated with building infrastructures and vehicle use. These studies verify that LCA can successfully quantify the environmental profile of alternatives in transport policy, if the relevant physical changes, e.g. vehicle travel distance and new infrastructures, are well-defined. However, before integrating LCA with other decision analysis methods for decision support, the study system, objectives, scopes, evaluation metrics and uncertainty handling need to be aligned.

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Conceptual framework for managing uncertainty in early design decision for sustainable aerospace
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In aerospace industry, life cycle assessment (LCA) is integrated into early design stage to predict environmental impacts for the purpose of sustainability. However, LCA's need for large amount of data and high-quality models yields uncertainty that influences its feasibility and the credibility of results. Although methods for analysing uncertainty are well developed in this field, data is scarce to evaluate uncertainty and the choice of appropriate methods for different types of uncertainty is not clear. Therefore, this study aims to investigate how to choose appropriate methods to handle uncertainty given available information within early design stage of aerospace. We firstly sketch a reference workflow by interviewing different stakeholders involved within design and manufacture processes of an aerospace company. The workflow includes functional requirement, risk management process and product life cycle, in which reqirement of information at each stage of the design process is defined. Then, we classify uncertainty within the workflow according to its nature and sources. Given different types of uncertainty, a decision tree is developed for choosing appropriate methods based on available information. As a result, integrating LCA within the traditional design process provides a growing resource of high quality information over product life cycle. Such "falling back" information is likely to become an additional beneficial driver for designers to optimise their product design in term of environment-friendly at the early design stage. The proposed decision tree provides a hierachical structure of methods to deal with uncertainties associated with different levels of information, so a specification of information needs to be explored within the workflow in order to provide a real state of knowledge. By considering uncertainty in eco-design workflow, we can use propagation method to evaluate uncertainty in output and identify "hot-spots" of uncertainties in inputs contributed to uncertainty in outputs, based on which data capture will be more effective and less costly. Therefore, managing uncertainty and unknowns is helpful for decision makers to make appropriate strategies in order to address the new approaches for improving eco-design within aerospace industry. In futher study, efforts should be undertaken to define specification of information in the design workflow and specify the proposed methods for uncertainty analysis in practical case studies.

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Plastics in the circular economy: LCA based indicators to guide the waste management policy in Flanders
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Recently, the European Commission has released its circular economy package to stimulate the implementation of a more resource efficient and circular economy. One of the key materials for which still a lot of progress can be made in this sense is plastics. Given its large masses dispersed in a huge array of applications, and their potential environmental impact when released in nature, many governments implement measures to improve the sustainability of plastic waste management. To monitor progress, suitable indicators are needed. At the level of products often LCA is used, however there are significant drawbacks for policy making such as its complexity in set-up and result and its missing link with the technical quality of waste streams. Therefore proper indicators should be sought that are based on LCA but better suited for policy support. This work will give an overview of 4 years experience in the practical implementation of the recyclability benefit ratio (RBR) indicator to the plastic waste management policy in Flanders. The presentation will go deeper into the advantages and disadvantages of the indicator, adaptations that have been made to make the indicator more advanced; e.g. for including quality or for including multiple recycling loops or open loop recycling. Results will be presented of three case studies in Flanders; one on domestic source separated plastic waste, one on plastic waste separated from WEEE and one on industrial waste from packaging production. Based on different policy questions, different scenarios will be compared such as closed loop recycling with incineration, the different options of open loop recycling starting from a certain waste stream, and the guidance of sustainable waste management schemes of waste streams with different qualities. Apart from methodological issues, attention will be paid to the way the Flemish government is using/can use the information delivered by these indicators to modify its subsidy and tax schemes.

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Unilever's framework for assessing impacts of bio-based raw materials.
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Rapid advances in technology are leading to the development of new, bio-based raw materials with potential sustainability benefits. Many of these materials rely on carbohydrates derived from agricultural crops. Unilever has developed a novel framework for the assessment of environmental impacts of bio-based raw materials. We frame the business decision-making context by differentiating four categories according to scale (supplier vs system) and timeframe (current impacts vs future impacts). Importantly, different methods and models are required to answer questions in the four quadrants of the framework. Whilst current and future production systems are often explored using attributional and consequential LCA approaches, respectively; the distinction may not always be helpful when assessing the impacts of company decision making. There may be opportunities to work at the intersection of these approaches, particularly when considering future production and impacts. We apply attributional Life Cycle Assessment for assessing current impacts, particularly occurring at smaller scale or within discrete geographical units. Land use change can be included, following the methods that look at historical changes in the land cover, e.g. following the principles of PAS 2050 standard. When describing current system scale impacts, we apply attributional LCA together with 'helper tools', e.g. modular extrapolation (MEXALCA). We are also developing regression-based mixed modelling techniques to advance extrapolation methods. Decisions regarding future supply of bio-based materials, require different, forward-looking modelling approaches. We have recently developed a method which we call Land Use Change Improved (LUCI)-LCA. The approach combines predictive, spatial modelling techniques with attributional LCA to assess greenhouse gas emissions, water consumption, erosion potential, nutrient leaching and biodiversity impacts of future raw material production and sourcing. Further scientific developments are required to extend the usefulness of our approaches, particularly for more informed decision-making related to future impacts of system scale changes. These include improving understanding of the impact of climate change on crops and more sophisticated land use change modelling to account for large scale increases in demand for bio-based raw materials.

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Downcycling vs Recycling of Construction & Demolition Waste: Combined LCA & LCC Analysis to Support Sustainable Policy Decision Process
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To ensure the environmental gain and the economic success of policy decision, policy making should be supported by decision tools which analyse (i) caused & avoided environmental impacts, and (ii) economic cost & benefits. Life Cycle Assessment (LCA) and Life Cycle Costing (LCC) are two well-known policy support tools which use similar goal and scope, functional unit and system boundaries. Thus, the results from both methodologies may be combined. However, due to the differences in methodology, metrics, temporal scale and weights, a combined environmental/economic analysis can lead to confusing and conflicting results and the integrated approach is seldom used to solve policy issues. The current paper focuses on the integration between LCA and LCC to stimulate Construction and Demolition Waste (CDW) recycling. Today CDW are recycled

mostly in low-grade application (filling material in road construction), also known as ***downcycling.*** The use of Recycled CDW in high-grade recycling (aggregates for concrete production) may decrease the amount of CDW to be managed, increase the economic value of the recycled material and reduce the quantity of natural aggregates employed in concrete production. The aim of the study is to provide a methodological framework where an integration between environmental and economic analysis can highlight the driving factors in CDW management. Results from both analysis can then be used in policy making to strive on the factors promoting sustainability and to limit on the ones representing a barrier to sustainable development. A LCA and a LCC analysis are performed anlysing four possible end-of-life scenarios for CDW: ***Landfilling***; ***Downcycling***; ***Recycling***; ***Selective Demolition***. The results highlight that reducing the impacts for natural resources depletion by increasing high quality CDW recycling, can sensibly reduce the overall environmental impact of the system. However the investment costs for the CDW recycling plant and the cost of selective demolition may be the economic factors limiting CDW high-quality recycling. Landfill tax, gate fees to recycling plant and tax on natural aggregates mining are the most effective elements for policy strategies in order to drive the CDW management system towards sustainable practices.

Do we have the right tools to identify emerging hazards and risks? (II)

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New approaches in regulatory science
D. Knight, ECHA-European Chemicals Agency
The REACH Regulation provides a high level of protection for human health and the environment. The legislator balanced the need to have information on the properties of chemicals with animal testing. New animal testing must only be done as a last resort. Registrants can make use of alternatives methods and approaches (i.e. Annex XI 'adaptations' of the standard information requirements). Such 'surrogate' data must be good-enough for classification and risk assessment: i.e. more-or-less equivalent to the standard animal study replaced. ECHA's Article 117(3) reports show that the most prominent alternative methods are read-across/categories and weight of evidence (WoE). Nevertheless, when examined many read-across and WoE cases are inadequate, i.e. poorly documented &/or with inadequate scientific justification. Note that ECHA's read-across assessment framework (RAAF) is a structured approach for examining read-across justifications. When the science is 'ripe' this has led to '3R's' changes in the standard information requirements. So far these are for 'lower-tier' endpoints important for the 2018 final registration deadline. ECHA's 2016 Topical Scientific Workshop on 'New Approach Methodologies in Regulatory Science' (where the acronym 'NAM' was coined) concluded that NAM could be useful for screening and prioritisation, but in general these approaches are not yet developed to draw regulatory conclusions on risk assessment and classification for higher-tier endpoints. Barriers to regulatory use of NAM are: (a) lack of understanding in judging the relevance of NAM evidence, (b) lack of quality standards on performance (to demonstrate robustness and reproducibility), (c) lack of common standards for reporting NAM evidence and (d) NAMs focus on toxicodynamics (e.g. reactivity) while the biokinetics (i.e. toxicokinetics) aspect is often missing. Improved read-across and WoE justifications for higher-tier (complex) endpoints offer the most promise for maximum impact in the short term. There is a clear need for (a) registrants to justify and document read-across (& WoE) cases systematically, (b) better techniques to address potential differences in TK ('biokinetics') between 'source' and 'target' substance in read-across and (c) a bigger 'toolkit' to provide evidence regarding toxicodynamics. The ECHA NAM workshop concluded that NAM evidence can be helpful to support 'read-across' cases.

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Industry perspective on new developments and gaps in hazard and risk assessment
S. Marshall, Independent Consultant; B. Hidding, BASF SE
This presentation focuses on two approaches for addressing the question of whether we have the right tools for identifying and assessing emerging hazards and risks. These are i) whether we have sufficient understanding of how ecosystems work and can then use this knowledge to focus hazard assessment, HA, and risk assessment, RA, and ii) whether we have an adequate HA toolbox and techniques to extrapolate their results to the field. i) Prospective regulatory RA schemes are designed as tiered frameworks where hazards are mostly assessed using a narrow range of standardised test methods that prioritise quality and ease of testing and cost effectiveness. Refinement of a RA may involve higher tier test methods to provide data of greater environmental and ecological relevance but are still mostly aimed at assessing toxicity to individuals of a single species. With the exception of rarely conducted multi-species studies, e.g. mesocosms, impacts on species in communities are not assessed. Rather, application factors are applied to account for uncertainties in extrapolation from single species laboratory tests to the field. There is little use of mechanistic understanding of how ecological communities work. Increasing application of ecological knowledge should increase the scope for identifying emerging hazards. For example, developing greater understanding of ecological interactions, e.g. via the description and modelling of ecological production functions, could provide a broader ecological basis for identifying impacts on key ecological entities that in turn could inform HA. ii) Even though current risk assessment schemes utilise a relatively narrow range of structural and functional endpoints compared to those represented in most ecosystems, there are still challenges in making some test methods fit for purpose in hazard identification or in RA. For example, there remain concerns over the acceptability of embryo tests and in vitro alternatives to the acute fish test as well as over chronic tests such as the Medaka extended one generation reproduction test. Experience of fate test methods such as the OECD 306, 308 and 309 tests indicates that technical enhancements are possible. If greater predictive capability is to be achieved by linking laboratory test data with advanced ecological and environmental modelling, then more reliable test methods are essential. These and other examples will be used to discuss whether standard test methods are fit for purpose.

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Hazard assessment methods in ecotoxicology: present and future
J. Tarazona, EFSA; M. Arena, EFSA - European Food Safety Authority / Pesticides; S. Barmaz, EFSA; D. Auteri, EFSA - European Food Safety Authority / Pesticides Unit
Hazard assessment methods have different roles as they can serve for: a) identification/grouping of substances with equivalent levels of potential harm/concern (e.g. aquatic classification categories) (b) identification of type of mode of actions causing effects (e.g. identification of endocrine disrupting properties), and c) setting endpoints for the use in the risk assessment. The development of ecotoxicological hazard assessment methods is a balance between the assessment needs clearly defined in the problem formulation and the technical limitations. The problem formulation play a key role first in the assessment/integration of information obtained at organism/sub organism level, in order to perform an ecotoxicological hazard assessment at population level and in the extrapolation from few species to the thousands of taxa and millions of species that should be covered by the hazard assessment. Technical developments in testing at organisms and sub-organism levels mostly adapt/mimic those from mammalian toxicology and are complemented by ecological-oriented testing, e.g. multispecies testing, population modelling and spatially explicit assessments. Technical limitations in the development of new ecotoxicological tests might be for example the need to reduce vertebrates sacrifice and high variability in the biological response. This presentation will review the current tools for conducting a hazard assessment, both at lower and higher tier including new approaches e.g. alternative methods to vertebrates testing (*in-silico*), omics; and use of population modelling. The available tools will be screened based on the needs for risk assessment and for supporting hazard based decision making in the regulatory frame. Finally, a new conceptual proposal for addressing environmental and ecological variability when assessing the ecotoxicological hazard potential of chemical substances will be presented. The proposal is part of the future developments under the EFSA 2020 strategy and focus on chemicals for which landscape structure is a key consideration regarding the identification and prioritisation of hazards for the risk assessment; this is directly relevant for pesticides and other agrochemicals, and can be extended to all chemicals with local or regional emissions into the environment.

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Mechanism-based toxicity testing and risk assessment: the EU-ToxRisk strategy
B. van de Water , Leiden University
The H2020 EU-ToxRisk project was launched in January 2016. The main aim of the project is to develop human relevant toxicity testing strategies that can be applied in a regulatory or screening context using only new approach methods (NAMs). These approaches will be based on mechanistic understanding guided by adverse outcome pathways (AOPs) that will be developed in the project. It will integrate advancements in cell biology, omics technologies, systems biology and computational modelling to define the complex chains of events that link chemical exposure to toxic outcome. The focus of this project is on two areas: repeated dose systemic toxicity, using the lung, kidney, liver and nervous system as examples of potential target organs; and developmental and reproductive toxicity. The project will directly address two complementary critical regulatory needs: 1) Pragmatic, solid "read-across" procedures between chemicals as the most important data gap filling and hence animal saving method at present, and 2) *ab initio* hazard and risk assessment strategies of chemicals with little background information. The EU-ToxRisk work plan is structured along a broad spectrum of case studies, driven by the cosmetics, (agro)-chemical, and pharma industry together with regulators and specialists from academia. Different, mechanism-based tiered test systems are integrated to balance speed, cost and biological complexity. The EU-ToxRisk strategy will be exemplified by sharing the status and latest outcome of one of the read across case studies.

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Panel discussion

21st century Ecotoxicology and Human toxicology: Applications and perspectives for the use of OMICs data (III)

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Multiscale connectivity - a high dimension biology approach to unravel the exposome

D.A. Sarigiannis, Aristotle University of Thessaloniki / Chemical Engineering

The exposome represents the totality of exposures from conception onwards, simultaneously identifying, characterizing and quantifying the exogenous and endogenous exposures and modifiable risk factors that predispose to and predict diseases throughout a person's life span. Unravelling the exposome implies that both environmental exposures and genetic variation are reliably measured in tandem and linked through mechanistic analysis of toxicity pathways rather than only phenotypically associated. To better understand the interaction between environmental exposure and disease, we need to; (a) capture the biological perturbations initiated by exposure to environmental stressors; and (b) identify which of these perturbations overcome the homeostasis barrier, resulting in observed alterations of the cell/tissue environment and eventually to pathologic phenotypes. Towards this aim, integrated exposure biology provides the methodological elements for the surveillance of changes at different levels of biological organization through the use of the full array of –omics and post-omics technologies including epigenomics. Starting from untargeted transcriptomics and metabolomics we proceed with joint analysis of biological processes induced by exposure to xenobiotics at the molecular level and of metabolic processes induced in parallel. This allows us to identify putative pathways of toxicity, which need to be verified by targeted multi-omics and functional assays. The connectivity approach was applied on data from a Europe-wide campaign on environmental and biological monitoring of a virtually ubiquitous mixture of volatile organic compounds and a complex mixture of polyaromatic hydrocarbons, which is typical of combustion products. The full array of –omics technologies were applied to samples of indoor air and dry blood spots and urine of exposed subjects from almost all European Union capitals. Following through to the biological processes that were perturbed by exposure to these airborne chemical mixtures we found that during acute (short-term) exposure signal transduction and mRNA transcription were modulated the most following an inverse dose-response function. When chronic (longer-term) exposure results were analyzed, protein metabolism, mRNA transcription regulation and cell proliferation and differentiation were the main mechanisms that were modulated.

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Stress response pathways in neuro-behavioural and reproductive toxicity of graphene oxide in C. elegans : Integrative multi-OMICS approach

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Graphene nanomaterials have attracted great research interest for their potential applications in electronics, energy, materials and biomedical areas. However, little information is available about their toxicity mechanisms. In this study, the potential hazard of graphene oxide (GO) was investigated in the nematode *Caenorhabditis elegans*, using an integrated systems toxicology approach. To gain an understanding of the underlying molecular mechanisms of GO toxicity, microarray, metabolomics, and lipidomics assays were performed, followed by an integrative pathway analysis. Results suggested the daf-16/FOXO pathway, drug metabolism, Fat metabolism, and MAPK pathway as potential toxic mechanisms of GO toxicity. To validate the detected pathways, functional genetic studies were performed, using loss-of-function mutants of genes of those pathways. Involvement of daf-16/FOXO pathway in neuro-behavioral and reproductive toxicity was validated by increased nuclear translocalization of the transcription factor daf-16 by GO exposure. Hence, we provide insights into the mechanism of toxicity of GO and suggest the direction for the further examination of graphene toxicity. The comprehensive approach allowed us to integrate all assay results in a consistent understanding of GO toxicity. Thus, this approach will serve as a proof-of-principle for the general suitability of multi-OMICS approaches for the elucidation of toxicity and stress responses, providing valid hypotheses which allowed to obtain unbiased knowledge. Keyword: Multi-OMICS, *C. elegans,* graphene oxide, Stress response pathway

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Molecular targets of narcosis and acetylcholine esterase inhibitors: Applications for chemical screening and identification using omics read-outs

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Environmental risk assessors are faced with an enormous number of chemicals that need to be evaluated for potential toxic effects. Modern technologies such as molecular read-outs can be useful for determining the class of an unknown chemical in a risk assessment. One class of chemicals which has proven difficult to identify with confidence are narcotics. There is no mechanism of toxicity established nor a way to screen for narcotics apart from predictive QSAR analysis. Based on previous findings in our lab we found that membrane proteins such as calcium channels could be impacted by exposure to narcotics. The objective of this work is to evaluate transcriptional and membrane protein targets during narcotic exposure in the model organism *Caenorhabditis elegans.* We also determined the ability of gene expression profiles to screen narcotic chemicals against specific-acting toxicants. *C. elegans* were exposed to a panel of 30 narcotic chemicals and transcriptional responses were measured by microarray analysis after a 24h exposure at 1/10 of the LC50. To determine specific membrane protein targets, we used the correlation values between log Kow and gene expression versus the correlations between membrane gene expression and all other genes in the dataset. These correlation matrices were used to classify neighborhoods of membrane genes (0% FDR and correlation >= 0.7), and significant results were put through gene set enrichment analysis (GSEA). Membrane protein targets include a number of ion channels as well as neurotransmitter receptors. Our results indicate that narcotics are able to consistently target membrane-bound proteins. As a follow-up analysis, we repeated microarray analysis of *C. elegans* exposed to a panel of 15 acetylcholine inhibitors at 1/10 of the LC50 for a 24h exposure. While LC50 results demonstrated a similar Kow-dependent response in both narcotic chemicals and acetylcholine inhibitors, transcriptional responses exhibit a strong difference between the two groups. However, predictive tools like GALGO are able to predict whether a chemical is a narcotic (polar versus nonpolar) or an acetylcholine inhibitor with up to 97% confidence. We will use the results of this follow-up work to determine specific genes for developing into a screening tool which can be used in a high-throughput manner to determine the potential class of an unknown chemical. These data will also further our understanding of the adverse outcome pathway for narcosis toxicity.

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The integrated use of omics technologies to understand mechanisms of Daphnia's stress response to cyanobacteria.

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Over the last decade, molecular technologies have evolved into robust high throughput platforms available to many scientists in a wide variety of disciplines. Implementation of these technologies in ecotoxicology and risk assessments have focused on mechanisms of toxicity and stress response on the gene level to explain effects at the organism level. Few studies, however, have used an integrated approach of a suite of molecular technologies, often due to the costs associated with them. Within this study, we have used genomics, transcriptomics and epigenomics to understand the mechanisms of cyanobacterial stress response in *Daphnia*. Cyanobacteria are a form of natural stress that are associated with the production of a wide range of toxins and are known to adversely affect many zooplankton species. Yet, the exact mechanisms of toxicity in *Daphnia* are still unclear. We have used cDNA microarrays to study gene expression patterns in different *Daphnia* clones in response to *M. aeruginosa*. We exposed different *Daphnia* clones with different sensitivities to *Microcystis*. We have used array CGH (Comparative genomic hybridization) to characterize the copy number variation at the DNA level between *Daphnia* clones with different sensitivities to *Microcystis*. We used whole genome bisulfite sequencing to characterize the cytosine methylation in response to *Microcystis* stress. \n We observed significant effects of *Microcystis* on gene expression that were genotype specific. In addition, we also observed significant differential regulation of trypsin isoforms showing a clear interaction effect between genotype and *Microcystis*. In addition, array CGH revealed 632 genes that showed significant copy number variation between clones sensitive to *Microcystis* and clones tolerant to *Microcystis*, including genes involved in oxidative stress and ribosome. Last, bisulfite sequencing indicated a significant differential methylation of 6 gene families including ribosomal proteins. Furthermore, a significant bias was observed for cytosines in coding regions. Indeed, differentially methylated cytosines were significantly more likely to occur in serine and threonine amino acid codons and were significantly less likely to occur in aspartic acid, glycine and histidine codons. Overall, our integrated results suggest a complex mechanistic response of *Daphnia* to *Microcystis* characterized by interactions between copy number variation, DNA methylation and gene regulation mechanisms. \n \n \n

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A multi-omics approach for identifying key events using the Adverse Outcome Pathway (AOP) framework

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An Adverse Outcome Pathway (AOP) is a framework which links knowledge of biochemical/physiologcal key events (KEs) to an adverse outcome (AO) at a biological level of organization relevant to risk assessment. KEs can be seen as deviations from a healthy state via a stress response that may be predictive of an AO and thus an indication of the potential health hazards of that chemical. In recent years the advances in 'omics technologies have opened new possibilities for discovering the molecular responses to chemicals in a non-targeted manner. Here we designed a three-phase experimental strategy that uses transcriptomics, metabolomics and targeted assays to attempt to identify and validate molecular KEs that are associated with an AO. Specifically, with a focus on regulatory ecotoxicology, we are applying this experimental strategy to *Daphnia magna* exposed to sublethal concentrations of the non-polar narcotic chlorobenzene that are known to chronically impair reproduction. During phase 1 of the study, *D. magna* were exposed to chlorobenzene at 0, 0.032 and 0.32 mg/L at high temporal resolution (11 timepoints between 0 and 8d for omics analyses, continuing to 21d for phenotypic endpoint measures) to identify key timepoints at which significant molecular pertubations are observed that may be indicative of a chronic AO; phase 2 will test the reliability of our phase 1 observations, while phase 3 will measure the dose-response characteristics of the molecular KEs and validate the discovery. The exposure to 0.32 mg/L chlorobenzene resulted in 25% mortality after 21d, no mortality occurred at 0 or 0.032 mg/L chlorobenzene and no mortality was observed during the timepoints selected for omics analysis. There was a significant reproductive impairment ($P < 0.01$) to *D. magna* at both 0.032 and 0.32 mg/L chlorobenzene identifying an AO that has potential ecosystem level effects. Metabolomics data met our QC criteria, and showed that the temporal metabolic effect through *Daphnia* development is greater than that induced by chlorobenzene with a marked difference in metabolic profile occuring when the daphniids reach ≥ 4 days old. Integration of the metabolomics and transcriptomic data will be used to identify the key timepoints on which to focus in depth to identify the molecular KEs that are associated with this AO.

Big data analysis of monitoring data: what questions can be addressed?

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The Edaphobase Nationwide Field Monitoring - A survey of Lumbricidae and Enchytraeidae assemblages in soil of different habitat types in Germany
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The aim of the Edaphobase database-project is to collect and provide ecological and taxonomic data on soil fauna. In the Nationwide Field Monitoring, a subproject of the current second phase of this project, various soil invertebrate organism groups from different habitat types in four regions of Germany have been sampled. The overall aims are to evaluate the usability of the database for nature protection issues and soil monitoring in general, to identify the optimum requirements of data to be included for such a purpose, as well to generate data for habitat types previously underrepresented in the database. The oligochaete (Lumbricidae, Enchytraeidae) assemblages were systematically surveyed using standard methods (ISO 23611-1, ISO 23611-3). The habitat types according to the 'German Red Data Book on endangered habitats' cover the land-use types forest, grassland and agricultural land, on both acidic and calcareous soils. Usually, three sites for each habitat type were sampled with 5 (Lumbricidae) or 10 (Enchytraeidae) replicate samples, each one sampled once in spring and once in autumn of 2014 or 2015. This design was used to examine a presumably wide range of ecological amplitudes. Thirteen earthworm species (incl. the first record of *Bimastos parvus* for Germany) and 75 enchytraeid species (plus ca. 20 new to science) were registered. Earthworm species richness and abundance and enchytraeid diversity are highest in grassland and lowest in farmed and coniferous forest sites due to tillage and low soil pH, respectively. Detrended correspondence analysis of earthworm communities reveals close similarity of sites of the same habitat type in 8 of 12 cases. The main drivers of habitat type-specific differences appear to be soil pH and C/N ratio: neutral to moderately acidic farmed land and grassland sites with a narrow C/N ratio have neutrophilic endogeic and anecic earthworm species. In contrast, (strongly) acidic woodland sites (deciduous and coniferous forests and plantations) with a wide C/N ratio have acido-tolerant epigeic species. Some enchytraeid species also reflect soil pH, even within a land use type (e.g., decrease of *Fridericia* species numbers with lower pH). Most diverse (35 species in 13 genera) are enchytraeid assemblages in plant species-rich, moderately acidic mesophilic montane grassland sites. Evaluation of the whole project data is still ongoing; especially community evaluations including all sampled organism groups is pending.

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Assessing the condition of forests exposed to climate change and nitrogen deposition by use of big monitoring data
W. Schröder, University of Vechta / 2; S. Nickel, University of Vechta / Chair of Landscape Ecology; M. Jenssen, Waldkunde-Institut Eberswalde
By example of Germany, a comprehensive and spatial explicit methodology for evaluating ecological integrity was developed. The approach integrates data on vegetation, chemical and physical soil conditions as well as on climate change and atmospheric deposition of nitrogen. Key component for evaluating ecosystem integrity is a classification of ecosystems containing information on ecological functions. Respective data covering 1961-1990 was regarded as reference. The assessment of ecological integrity relies on comparing a current or future ecosystem status with the reference. Whilst current ecosystems were quantified by measurements, potential future developments were projected by geo-chemical soil modelling and data from a regional climate change model. The ecosystem types were referred to the potential natural vegetation and mapped additionally using geo-data on current tree species coverage and land use. The current ecosystem types were related to geo-data (elevation a.s.l., soil texture, air temperature, humidity, evapotranspiration, precipitation 1961-1990) by Classification and Regression Trees. The relations determined by this were applied to the above mentioned geo-data and then used to map the spatial pattern of ecosystem type clusters for 1961-1990. Then, the climate data 1961-1990 were replaced by results from a regional climate model for 1991-2010, 2011-2040, and 2041-2070. Accordingly, for each period one map of ecosystem type clusters were produced and evaluated with regard to the development of areal coverage of ecosystem clusters across time due to climate change. This evaluation of structural aspects of ecological integrity in terms of bio-geographical coverage on the national level was added by projecting potential future values of indicators for ecological functions at site-level. This was achieved by using the Very Simple Dynamics soil modelling technique using the above mentioned climate data and two scenarios of atmospheric nitrogen deposition as input. The results were compared to the reference and enabled evaluating site-specifically ecosystem integrity across time.

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Disentangling the effects of low pH and metal mixture toxicity on macroinvertebrate diversity
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One of the primary goals of biological assessment of rivers is to identify whether contaminants or other stressors limit the ecological potential of running waters. Quantitative relationships between species richness and environmental gradients are useful to better understand biodiversity patterns. However, describing such relationships for a single environmental gradient is not straightforward, because species richness is typically dependent on a multitude of environmental gradients. Many studies have focused on the effects of pH and high metals concentration on freshwater macroinvertebrate community but, due to data limitation and the lack of evaluation tools, the ecological effects of metals mixture in streams are less studied and still unclear. We address an old question: is it the low pH or the metals that are deleterious for stream ecosystems? With new tools, we can achieve improved understanding of the true importance of the two stressors. The accumulation of metals in organisms was predicted using an equilibrium speciation model developed by the US Geological Survey to help understand how the changes in water quality influence the ecology of these systems. Because most metal-contaminated streams were affected by mixtures of metals (Al, Cd, Cu, Pb, Zn), we compared a concentration addition and an independent action approach of aqueous metal toxicity. Our study quantified the limiting effects of pH and chronic metal mixtures toxicity for macroinvertebrate community richness. We verified that current environmental quality standards for metals are protective of aquatic biodiversity and proved that pH has a direct limiting effect on richness and it does not only act via modifying the availability and the toxicity of metals. These questions were applied to a dataset spanning two continents with diverse geological features and ecosystems, providing a broad basis for understanding how physico-chemical conditions limit global freshwater biodiversity.

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Compilation and analysis of monitoring data from eutrophied lakes across Europe: experiences from EU projects
J. Moe, Norwegian Institute for Water Research (NIVA) / Section for Catchment Processes; A. Lyche Solheim, K. Austnes, Norwegian Institute for Water Research NIVA
Following the implementation of the European Water Framework Directive, large amounts of monitoring data from thousands of water bodies across Europe have been compiled and analysed in several EU research projects. These projects have typically focused on environmental stressors such as eutrophication, acidification and hydromorphological alteration, but to a less degree on environmental toxicants. Here we will report on our experiences with compilation, management and analysis

of monitoring data from a series of EU projects (REBECCA, WISER, REFRESH and MARS) addressing ecological effects of eutrophication in European lakes. We will highlight achievements from the use of these large-scale datasets for fundamental and applied research as well as the usefulness for river basin management. Examples will be given from the ongoing project MARS, targeting multiple stressors such as eutrophication and climate change for lakes at the European scale. We will address the challenges related to compilation and analysis of large multi-national environmental datasets, and provide recommendations based on our experience. We will also inform about the publicly available monitoring data in WISE (Water Information System for Europe), including hazardous substances as well as biological quality elements in rivers and lakes, and the potential for use of these data in research.

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State-of-art and SWOT-analysis of existing fresh water monitoring approaches
M.G. Vijver, CML Leiden University / Conservation Biology; E. Hunting, S. De Jong, CML Leiden University; W. Peijnenburg, RIVM / Center for Safety of Substances and Products; E. Smit, RIVM / Centre for Safety of Substances and Products; C. Bodar, National Institute for Public Health and the Environment RIVM
Results from national and regional monitoring programs and authorities at river basin district scale are the most important ways of getting an overview of Europe's water quality and the pressures affecting this quality. The most prominently implemented schemes are chemical monitoring with its ultimate aim to evaluate the clean sustainable society, and the ecological monitoring with its ultimate aim of providing the evidence of a non-toxic environment. The assessment of the environmental state of European surface waters comprises the collection and aggregation of a huge amount of information. Reliable, high quality information about the chemical and ecological state of surface waters is essential for water management and for improving the environmental quality of Europe’s waters, especially in relation to the Water Framework Directive [1]. Merging both types of monitoring allows for meeting the challenge of quickly assessing whether substances and mixtures will lead to ecological effects. With new techniques becoming available, the status of the environment can more efficiently be evaluated: from ecotoxicogenomic changes induced by substances to the use of so-called bioassay batteries ("the canary in the mine") and eco-epidemiology. The central - scientifically significant - questions are: are we looking at the right indicators and effects, are we efficiently taking the appropriate actions, and do we do this in the most cost-effective manner? What do we actually want to know and how should we measure? In this presentation we give an overview of current approaches used for chemical and ecological monitoring as allowed under the Water Framework Directive [1], and we provide a Strengths Weakness Opportunity and Threats analysis.

Organic micropollutants in the environment: analytical challenges and engineering innovations (II)

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Impact of cleaning procedures and type of materials on the measure of 20 bisphenols in surface water samples
c. ferret, INERIS; P. Rostkowski, NILU Norwegian Institute for Air Research; B. Lepot, F. Botta, INERIS
BPA is one of the most produced and used chemicals worldwide, even though it was a recognized synthetic estrogen. About 70% of BPA production (3.4 million tons per year) is used to produce polycarbonate plastics used in a variety of common products. Consequently, during sampling operation in river, if the operator uses a plastic bucket, a risk of sample precontamination can be observed. This study will give some warnings on the cleaning steps and on which material needs to be used during water sampling for environmental monitoring of bisphenols. Material testing and on-field sampling were performed by INERIS. 5 different sampling methods were used on a reference site and in laboratory: direct sampling in the river; indirect with a plastic bucket, a horizontal PFTE bucket, a telescopic rod and vial and a new bucket (uncleaned). Two types of tests were performed on these 4 types of sampling material : A) different rinsing process of bucket/bottles before sampling in laboratory. This process was repeated twice and samples were analysed after the 1st and after the 2nd process B) on-site sampling with the cleaning process validated during the 1st test in laboratory. A new with plastic bucket (used immediately after buying and without cleaning) was also tested. Analyses were carried out at NILU on 20 bisphenols. Firstly, this study displays a risk of precontamination not for all bisphenols, but for 9 out of 20 (essentially Bisphenol A and bisphenol BP). This study suggests a potential contamination of the sample by bisphenol if the sampling operator do not apply a multiple and correct cleaning procedure on the laboratory before field operations. Authors also observed that samples collected with a non-rinsed plastic bucket were highly contaminated, especially for bisphenol A. Hovewer, in this study concentrations were very low (high sensibility of analytical method) but still very far from PNEC threshold (0,2 μg/L for Bisphenol A).In conclusion, AQUAREF reccomends a strict cleaning procedure and few field blanks before to collect field sample for bisphenols investigations.

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Enantiomeric fractionation for quantitative assessment of anaerobic biodegradation rates: The case of climbazole
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An efficient chiral liquid chromatography - high resolution - mas spectrometry method has been developed for the determination of climbazole (CBZ) enantiomers in wastewater / sludge. The obtimized analytical method has been validated with good quality parameters including resolution > 1.1 and method quantification limits down to the ng/L range. On the basis of this newly developed analytical method, the stereochemisttry of CBZ was investigated over time in effluent / sludge biotic and steril microcosms under anaerobic dark and light conditions and in influents and effluents of five different subsurface constructed wetlands (CWs). CBZ stereoselective degradation was exclusively observed under biotic conditions, confirming the specificity of enantiomeric fraction variations to biodegradation processes. CBZ was always biotransformed into CBZ-alcohol due to the specific and enantioselective reduction of the ketone function into a secondary alcohol function. This transformation was almost quantitative and biodegradation gave a good first order kinetic fit. We investigated the possibility to apply the Rayleigh equation to enantioselective processes by replacing the isotope ratio by the enantiomer ratio for quantitative biodegradation assessment of CBZ in biological treatment processes and in receiving surface water or soil. The results of enantiomeric enrichment pointed the way for a quantitative assessment of in situ biodegradation processes due to a good fit ($R^2 > 0.98$) of the anaerobic CBZ biodegradation to the Rayleigh dependency in all the biotic microcosms and in CWs. These results demonstrate that enantiomeric fractionation constitutes a very interesting quantitative indicator of CBZ biodegradation under anaerobic conditions. The enantiomeric fractionation tool does not imply to achieve a mass balance of the contaminant, which constitutes a relevant advantage over the conservative tracer approaches. A prediction of the extent of biodegradation is also allowed as fars as the enantiomeric enrichment factor is known.

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Chlorination and chloramination of phenazone-type drugs and metabolites
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Phenazone-type pharmaceuticals, such as aminopyrine, metamizole, phenazone (Phe) and propyphenazone (PrPhe), are widely used analgesics. Aminopyrine and metamizole are extensively metabolized in the human organism to aminoantipyrine (AA), 4-acetamidoantipyrine (AAA) and 4-formyl-aminoantipyrine (FAA) [1, 2]. These pharmaceuticals and metabolites have been detected in ng L^{-1} concentrations in environmental water bodies and in resources used to produce drinking water [3]. In this study Phe, PrPhe, AA, AAA and FAA were treated with chlorine and with monochloramine under laboratory conditions. Chlorination showed to be more effective in phenazone–type pharmaceuticals depletion (with half-lives, considering pseudo-first order kinetics, below 30 s at pH 7) than chloramination (with half-lives above 6 h at pH 7). The influencing factors on the removal of these compounds by chlorination were explored at chlorine dosages ranging from 1 to 10 $\mu g\ mL^{-1}$, pH values ranging from 5.7 to 8.3 and bromide concentration ranging from 0 to 200 ng mL^{-1}. On the other hand, during chloramination pH values influence was also studied in the range 5.7 – 8.3. Transformation products (TPs) were tentatively identified by using liquid chromatography (LC) and high resolution mass spectrometry (HRMS) with a quadrupole –time of flight (QTOF) system. The transformation path of these drugs and metabolites consisted mainly of halogenations, hydroxylations and dealkylations. Finally, a preliminary evaluation of the TPs was performed using quantitative structure-activity relationship (QSAR) tools, where it was observed that some TPs are of higher concern than precursor compounds. References [1] Levy, M., Zylber-Katz, E., Rosenkranz, B., 1995. Clinical pharmacokinetics of dipyrone and its metabolites. Clin. Pharmacokinet. 28, 216–234. [2] Agúndez, J.A.G., Marinez, C., Benítez, J., 1995. Metabolism of aminopyrine and derivatives in man: in vivo study of monomorphic and polymorphic metabolic pathways. Xenobiotica 25, 417-427. [3] Wiegel, S., Aulinger, A., Brockmeyer, R., Harms, H., Löffler, J., Reincke, H., Schmidt, R., Stachel, B., von Tümpling, W., Wanke, A., 2004. Pharmaceuticals in the river Elbe and its tributaries. Chemosphere 57, 107-126. *Acknowledgements* - The authors acknowledge the support of Xunta de Galicia (EM2014-004, GRC2013-020) and FEDER/ERDF.

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Technical and economic assessment of real-time control strategies for micropollutant removal by ozonation at pilot-scale
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Ozonation for municipal wastewater effluent has shown to be a promising technology for micropollutant (MP) removal but the basic control strategies currently applied (e.g. flow-based) result in sub-optimal ozone dosing. Optimal dosing lowers, however, operational costs and minimizes by-product formation.

Additionally, individual MP analyses are time, cost and labour intensive which enhances the need for complementary more readily available (online) and less expensive surrogate measurement techniques. The potential value of simple surrogate models, e.g. correlating the decrease of UV absorption at 254 nm (DUVA254) to MP removal, has been recognized and novel empirical models have been suggested based on lab-scale experiments. Reliable sensors are already broadly commercially available containing the UVA254 measurement which can be easily integrated with the recently developed correlation models. In this work, recently developed correlations were applied at pilot-scale as a proof-of-concept and to investigate/evaluate the behavior when real-scale events and/or effluent variations occur. Through monitoring such behavior over several months, the goal was to acquire new insights for a wider application of these control strategies. A small underestimation of MP removal (± 10%) was observed during online control of the ozone pilot, probably due to minimal fouling of the online sensors during experimental trials. To make a more comprehensive evaluation, the control strategy based on the DUVA254 was compared with two more conventional alternatives: flow-based (i.e a fixed O_3 dose expressed as mg O_3 L^{-1}) and load-based (i.e. a fixed O_3:DOC ratio using the UVA signal only before ozonation of the effluent). Operational expenses could be reduced with 10 to 25 % depending on the effluent compositions, weather conditions and applied control strategy. The largest cost saving was obtained compared to the fixed ozone dose approach (i.e. flow-based control). The O_3:DOC ratio strategy resulted in more or less similar costs as the DUVA254 approach but would however have led to periods with both an ineffective micropollutant removal and a clear overdose of ozone. Changes in matrix reactivity are clearly not captured, in contrast to the DUVA254 strategy for the same period of pilot operations. Consequently, a more accurate ozone dosing at all times will result in a more reliable micropollutant removal and lower by-product formation.

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Poster spotlight: WE091, WE092, WE093, WE094

Environmental consequences of oil and gas extraction and transport

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Differences in exposure media profile and toxicity following preparation of water-accommodated fractions along an energy continuum
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The evaluation of oil toxicity, particularly in a spill scenario, is complicated due to the complex physical, chemical and biological environment in a given situation. Oil is a complex mixture of hydrocarbons whose fate and toxicity is highly affected by composition and chemical/physical weathering processes that result in dissolution, dispersion, and formation of micron and submicron-sized oil droplets. Understanding the contribution of dissolved and undissolved-phase hydrocarbons (DPH and UDPH respectively) to toxicity is important as the majority of analytical methods combine the contribution of these groups due to the limitation of the extraction procedures used in preparing samples prior to analytical characterization. It is possible to produce a solution containing hydrocarbon components at their maximum solubility using "controlled energy" mixing techniques, but it will contain a limited quantity of UDPH materials. Rather than continuing to try to develop an empirical technique to produce UDPH-free water accommodated fractions (WAFs), we assessed the contribution of the UDPH fraction by assessing a series of solutions prepared over a range of mixing energies that result in a range of UDPH content. At the highest energy mixing conditions UDPH contribution will be greatest while in the lowest mixing energy the resulting solution will be mostly dissolved. A series of XWAFs (where X relates to the energy level) were generated using standard protocols with non-weathered oil, along an energy continuum; No Energy (*NE*, passive diffusion through silicone tube), Medium Energy (*ME*, 20-25% vortex), Intermediate Energy (*IE*, 40-50% vortex), and High Energy (*HE*, low speed on blender [~15,000 rpm] for 30 seconds). These XWAFs were physically and chemically characterized with particle counting, 3D EMM Fluorescence, and analytical chemistry, and used as exposure media in 14-d semi-chronic static renewal (larval *Cyprinodon variegatus*) and 48-h static (*Americamysis bahia*) toxicity tests. There were considerable differences in exposure media profiles, which were subsequently reflected in the toxicity results. The HEWAF preparation was more toxic to both species and had greater TPAH concentrations characterized by the presence of UDPH and higher molecular weight aromatics, than the NE-, ME-, and IEWAF preparations. These results demonstrate the importance of characterizing exposure media, and the ability of varying preparations to accurately mimic real-world samples.

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Development of a Generic Exposure Scenario for substances used in high volume hydraulic fracturing fluid products under the REACH regulation
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The European Commission Recommendation (2014/70/EU) on minimum principles for high-volume hydraulic fracturing includes the requirement that substances should refer to "hydraulic fracturing" in their REACH registration dossier, if they are to be used in such applications. Based on this recommendation and in line with REACH requirements, it was recognised that specific use descriptors would be important for each dossier, and that the development of models or more relevant Specific Environmental Release Categories (SpERCs) will benefit environmental exposure assessments. A team of industry representatives was established that includes various oil & gas operators and service companies. The Hydraulic Fracturing Exposure Scenario Task Force worked on the development of a Specific Environmental Release Category (SpERC) that is based on realistic data and assumptions for high volume hydraulic fracturing as an intended use. The background document details the supporting information used in the development of the generic exposure scenario. Each phase of the hydraulic fracturing process, such as formulation, the fracturing operation and the management of flowback were reviewed. Industry has provided conservative, but realistic parameters that supports a 1st tier environmental risk assessment for the use of substances in high volume hydraulic fracturing within the framework of the REACH legislation and has been established that, if the controls specified or those offering similar levels of mitigation are in place, emissions are minimal.

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Oil Sands: integrate natural processes in hazard assessment
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One of the largest Cretaceous mixture of sand, clay, and bitumen deposits (also referred to as oil sands) in the world occurs in the Athabasca Basin in north-eastern Alberta, Canada. Bitumen, which is a heavy, viscous form of crude oil, is composed of a complex mixture of hydrocarbons, hetero-organics and metals. The release of contaminants from oil sands deposits can occur naturally through weathering and fluvial geomorphological processes and/or from anthropogenic activities such as mining and upgrading processes. The Athabasca river and associated tributaries which flow through geological regions containing bitumen deposits (ie., the McMurray formation) experience river bank and bed erosional processes that result in bitumen containing entering the rivers. The main objective of this study was to evaluate the acute and chronic ecotoxicological effects associated with the erosion of bitumen containing soils that naturally enter the river systems through fluvial geomorphological processes. Ecotoxicological tests were conducted using parental geological material collected from the river banks in the oil sands mining area (Alberta, Canada), in four different locations. Samples differed from each other mainly in terms of texture and proportional bitumen content due to the differences in the specific geological formations in each river basin. The toxicology of solid bitumen samples was assessed using a solid phase MICROTOX® test (which contained the bacteria *Vibrio fischeri* as the test organism), and a chronic 28-days Partial life-cycle test with aquatic midge larvae (*Chironomus riparius)*. Bacteria were found to be more sensitive to bitumen contaminant exposure compared to chironomid. A significant decrease on larvae growth and delay in emergence time was observed in *C. riparius* in treatments having high bitumen content. The use of solid samples combined with a suite of representative species provides a comprehensive and ecologically relevant approach to assessing the toxicological effects of natural oil sands exposure arising from bank erosion-related processes. The study emphases the need to better discriminate natural processes from anthropogenic sources (mining related activities) of surface and groundwater contamination in oil sands areas.

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Fate and effects of oil in ice
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The Arctic Oil Spill Response Technology Joint Industry Programme had the goal to advance arctic oil spill response strategies and equipment as well as to increase understanding of potential impacts of oil on the Arctic marine environment. In 2013 a comprehensive review of investigations into environmental consequences of spilled oil and oil spill response technologies in the Arctic marine environment was performed by a multi-disciplinary team of experts. This review indicated that there is a significant science base for oil spill response decision-making in the Arctic already available and also listed suggestions for follow-up work to enhance the science-base for Arctic Net Environmental Benefit Analysis. To follow up on the recommendations a long-term mesocosm experiment was executed to improve the scientific knowledge of the fate and biodegradation of oil and oil spill response residues in ice, as well as the environmental effects to ice associated ecology. Eight mesocosms were installed in the sea ice of the Van Mijenfjorden in Svea, Svalbard in January 2015 and remained in place until July 2015. Oil was introduced into two

mesocosms to follow natural attenuation. In two other mesocosms oil mixed with dispersant was introduced and another pair contained burned residues mimicking an in situ burn response. The two remaining mesocoms served as controls. The study was designed to monitor the fate, behaviour, persistence and biodegradation of the oil in ice together with the impact on the microbial communities during winter and spring. Under-ice phyto- and zooplankton communities were sampled and monitored for effects. The following parameters were studied within the water column, through the ice layer and within the water-ice interface:\n • Chemical composition of the oil\n• Bacterial populations and oil degrading microorganisms\n• Microbial activity and biodegradation activity\n• Zooplankton - survival, feeding and reproduction (under ice)\n• Ice algae primary production Similar exposures were replicated in the laboratory to measure the sensitivity and the resilience of polar cod. The studies and additional modelling activities have improved our understanding of what happens to oil once frozen into ice, how microbiology is reacting to oil in ice and what the exposure potential is of the ecology associated with the ice. This helps the response community in selecting a combination of response strategies that minimises the effects to people and the environment.

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Poster spotlight: WE144, WE145, WE146, WE147

Environmental risk assessment in time and space - To boldly go where no man has gone before

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Molecular to Landscape, the integration of adverse outcome pathways into a regional scale pesticide driven risk assessment for Chinook salmon in Puget Sound watersheds.
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A challenge in environmental toxicology and risk assessment has been the extrapolation from the molecular interactions within an organism to the landscape scale effects on population dynamics and community structure. We have now demonstrated the potential to do so in a regulator manner by building adverse outcome pathways into cause-effect models for ecological risk assessment at landscape scales. The model framework is derived from the Bayesian network relative risk model that has been used at a variety of scales, from watersheds to continental regions. Our case study is the risk calculation incorporating the use of organophosphate pesticides in four watersheds in the state of Washington (USA) estimating the effects upon populations of Chinook salmon. For example, data on the probability distributions of the occurrence of malathion during several years, both concentration, location and time of year, are fed into an adverse outcome pathway that estimates acetylcholinesterase inhibition. The percent inhibition then informs nodes on swimming speed and other factors that impact survivorship. In a parallel sequence nodes that describe water and habitat quality are constructed. These nodes also include the effects on the pesticides on food availability. The pesticide pathway and ecological pathway are combined into nodes that describe fry length and juvenile density, two factors that are known to be critical in estimating the Chinook populations. Finally, the population dynamics over a 50-year period are estimated using age structured models. First simple single population models will be used followed by metapopulation estimates. Calculations are performed and entropy analysis, a type of sensitivity analysis, points to the most influential variables in the model. Tools to estimate the effects of mixtures are under construction. Previously, it has been demonstrated that BN-RRM models can be modified to incorporate management alternative and externalities such as climate change. As has been demonstrated previously by Landis et al (2017) risk assessment can be integrated into an adaptive management framework for decision-making that incorporates a variety of management options, can point to data requirements, defines monitoring goals, and can include externalities such as climate change.

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Integration of aquatic exposure and effect models at landscape scales: an example case study and some conceptual considerations
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Current environmental risk assessment (ERA) of pesticides in aquatic systems is rather generic. This means, on the effect side of risk assessment generic threshold values are derived from laboratory or semi-field trials, and on the exposure side simulation models are used to calculate proxies for maximum and average concentrations for spatially restricted, selected locations across Europe. This approach was a big step forward for ERA two decades ago, but in the meantime the science behind regulatory risk assessment, data availability and mechanistic understanding of fate and effects of pesticides has improved. Nowadays, the ERA of pesticides could step forward from its current rather generic approach towards a landscape-scaled, spatially explicit risk assessment. Considering the landscape scale in the ERA for pesticides has three main advantages: (1), local environmental conditions can be considered for risk assessment decisions; (2), the expected impact on the environment and hence the consequences of risk assessment decisions across European agricultural landscape can be calculated instantaneously; and (3), risk assessment can be better linked to specific protection goals and risk management and risk mitigation measures can be integrated into a spatially explicit context. To enable this approach, exposure and ecological models need to be linked to each other within a spatially explicit landscape context. In this contribution, we outline some general considerations about conceptual and technical concepts and show an example case study for such a spatially explicit risk assessment. In the example case study, the exposure model CASCADE-TOXSWA was used to calculate fate processes for the insecticide cypermethrin and the fungicide fluazinam following application to potatoes for a typical Dutch agricultural landscape, that consists of a ditch system with in total 65 km length and about 10 km^2 size. Exposure concentrations in water were linked to a toxicokinetic-toxicodynamic model which translated the exposure into expected mortality for the water louse *Asellus aquaticus* and the mayfly *Cloeon dipterum*. Population level effects for different subparts of the catchment were calculated, and population dynamics were simulated including the recovery of the populations in a single-species approach. In addition, generic ERA was performed by linking FOCUS TOXWSA simulations for the representative Dutch ditch scenario to the same TK/TD and population modelling.

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Individual-based modelling as a population-level risk-assessment tool in the identification of emerging pollutants in European catchments
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In the context of the SOLUTIONS project [1], we explore the use of individual-based models (IBMs) for population-level risk assessment (RA). The models are to be applied for a large number of substances and a large number of locations (>30,000 sub-catchments in the major European catchments), and for a set of sensitive species. Water concentrations are obtained from chemical fate modelling (STREAM-EU) [2]. Given the computational demands, the IBMs need to be simple but able to take into account impacts on survival, growth and development, and reproduction, to potentially exploit all available information on the sensitivity of selected species to the tested substances. Mixture toxicity should be incorporated, as many of the substances are in isolation unlikely to exceed thresholds for lethal or sublethal effects. As an integrative population-level endpoint we used the chemical's impact on projected population growth rate l, from which an indication of the population's recovery capacity can be derived. Alternative dose-response (DR) relationships can be applied, depending on the data available, a linear DR (requiring only an EC50 value), linear with NOEC (EC50 & NOEC) and log-logistic (EC50 & shape parameter). Response addition was assumed to assess the effects of mixtures. As a first test, the model was run for 4 aquatic macro-invertebrate, "sentinel" species, *Daphnia magna*, *Chironomus riparius*, *Gammarus pulex* and *Aselsus aquaticus*, evaluating WFD priority substances. The impact was quantified by the ratio of daily population growth rates under exposed and reference situation (l_{exp}/l_{ref}). Results comprise the relative population growth rates for >30.000 subcatchments in Europe for each species and a number of WFD priority compounds. It appeared feasible to run such a large number of simulations and to quantify risk per species at large catchment levels. Apart from such risk maps, distributions of growth rate effects can be calculated, from which statistical indicators can be derived to summarize risk. [1] Brack, W., et al., *The SOLUTIONS project: challenges and responses for present and future emerging pollutants in land and water resources management.* The Science of the total environment, 2015. **503-504**: p. 22-31. [2] Lindim, C., J. van Gils, and I.T. Cousins, *A large-scale model for simulating the fate & transport of organic contaminants in river basins.* Chemosphere, 2016. **144**: p. 803-810.

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Ecological scenarios for prospective risk assessment of aquatic ecosystems
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Identifying the most sensitive species in a given ecosystem is a key challenge to risk assessors. This study explores a methodology that enables pinpointing these species based on their biological traits and use them for the construction of ecological

scenarios. It is suggested that interspecies variation in sensitivity to toxicants increases with increasing toxicological specificity of the toxic mode of action (MOA). Therefore narcotic compounds, which have a nonspecific MOA, were tested. First existing data on MOA and chemical structure, specifically the subdivision between esters, polar or non-polar compounds, was extracted from a publicly available database. By applying this division, remaining differences in toxicity are hypothesised to be mainly due to differences in toxicokinetic parameters. Next, the relative sensitivity of invertebrates to the three narcosis groups was calculated. Biological trait information was related to the calculated relative sensitivities, allowing the development of multiple regression models for the prediction of the sensitivity of untested species. The best model was used to predict the sensitivity of the communities of the Water Framework Directive (WFD) ecoregions for rivers and lakes. The average sensitivity of the most sensitive 25% of the species was used to identify potentially most sensitive regions. Results show that the sensitivity of invertebrates to narcotics is indeed partly related to the used traits, and therefore the sensitivity of untested species can be predicted by applying the traits-based approach. Different WFD ecoregions showed little variation considering their sensitivity to narcotics, which might be due the large spatial scale of the regions. Each region is able to sustain such a diverse species composition due to the presence of various habitats that average traits composition of the regions are more or less similar. Further research should focus on improving ecotoxicological-, traits- and MOA-databases, on exploring different spatial scales for scenario development, and on linking of developed scenarios with ecological models.

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Influence of local habitat connectivity and isolation on plant community resilience
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Semi-natural grasslands are important for maintaining biodiversity in agricultural landscapes. Due to land-use shifts, the intensification of agriculture and other socio-economic developments, these grasslands often suffer from a loss of connectivity and increased isolation, limiting the seed dispersal and thus the exchange of seeds between communities. Besides species composition, seed input and seed banks are important factors for the resilience capacity of grasslands. Highly fragmented off-field habitats in immediate proximity to the agricultural field might experience stronger impacts, if there is not enough (external) seed input of undisturbed areas or a seed bank to buffer potential effects of herbicides on the plant community. In our approach we used the plant community model IBC-grass to analyze the resilience capacity of a plant community with different degrees of isolation, represented by different seed input scenarios, and distances to an agricultural field to account for different disturbance levels. Based on an extensive literature review, we parameterized IBC-grass for a typical herbaceous field-edge community in Europe. Due to the individual-based approach of IBC-grass, we were able to address potential herbicide effects on plant individuals and upscale individual-level effects to plant community dynamics. We extend the model by different external seed input and seed bank scenarios. The simulated exemplary herbicide effects biomass, survival and establishment of plants, ranging from high effect levels to low effect levels to account for the distance to the agricultural field. Our preliminary results indicate that the degree of isolation (as defined by level of new seed input) influences the resilience and recovery capacity of grassland communities in agricultural landscapes. Plant community models like IBC-grass could be a helpful tool in this context; particularly to put results from small-scale experiments into context.

Microplastics, nanoplastics and co-contaminants: Fate, effects and risk assessment for biota, the environment and human health (I)

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Associations of nanoplastic with freshwater algae traced using a novel fluorescence assay
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Globally, huge quantities (approximately 8 million tonnes) of plastic are produced every year and there is increasing public concern on the impacts of plastics and their degradation products on aquatic ecosystems. Plastic debris in aquatic environments are subject to an array of abiotic processes that weather and degrade them, leading to the formation of micro- and nano-plastics. There has been a growing body of research on the biological effects of microplastics in the environment, but to date little research on their smaller counter parts – nano plastics. Furthermore, much of this research has focused on marine environments with far less information available for freshwater systems. Here we address the hypothesis that the entry routes of nanopolystyrene into a freshwater food web will be influenced by particle ageing. In the long run we are investigating transfer between three organisms, representing a simple food web, which includes an algal species (*Chlorella*), a crustacean (water flea, *Daphnia*) and a fish (3 spined stickleback) as a model system. Algae, are ubiquitous within freshwater environments and it is likely that they will accumulate micro and nano plastics from the water, not only via cellular uptake but also via sorption onto their cell surfaces. Algae in turn will be consumed by freshwater crustaceans such as water fleas and in turn fish. Our first challenge was to quantify the uptake of nanoplolystyrene into *Chlorella*. To do so, we developed a novel microplate fluorometric assay to measure the tissue burdens in *Chlorella* exposed via the water to fluorescently-doped nano-polystyrene (PNP). In brief, PNP in algal cultures were separated from the aqueous phase PNP using centrifugation, and the algae-PNP loading was measured by extracting the fluorescein derivative dye, which was then quantified using a fluorescence plate reader. Using this assay, our early experiments have examined associations of PNP with algae and how they vary with time of exposure and PNP exposure concentration. We are now expanding this work to consider the effects of surface modifications and weathering processes on these relationships. Results from these initial studies and the implications for trophic transfer will be presented and discussed. Evaluating the effects of environmental influences on the bio-reactivity of degraded plastic will help to better inform us of the true risk to organisms within aquatic environments. This project is funded by a **NERC grant.**

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The presence of microplastics affects toxicity of the pesticides deltamethrin and dimethoate to Daphnia magna differently, based on differing hydrophobicities
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Anthropogenic activities are having enormous impacts on today's ecosystems, with microplastics and pesticides both shown to have adverse effects. However, there have been few studies investigating the interactions, dynamics and kinetics between these pollutants. Hydrophobic organic chemicals (HOCs), including many pesticides, are recognised as having high lipophilicity (high log Kow, octanol:water partition coefficient), and have been shown to bind preferentially to plastics over natural sediment. The aim of this study was to investigate how the presence of microplastics will affect the toxicity of pesticides to *Daphnia magna*, based on binding affinity. The pesticides selected for this studywerethe pyrethroid insecticide deltamethrin (high log Kow = 6.2) and the organophosphate insecticide dimethoate (low log Kow = 0.7). *Daphnia magna* were exposed to these chemicals with and without microplastics (1μm polystyrene beads) at a fixed concentration of 300 000 particles per ml. Microplastics reduced the toxicity of deltamethrin to *Daphnia magna*, with time a significant factor influencing toxicity when microplastics were present within the system. This is likely to be due to the binding of the hydrophobic chemical to the plastics, reducing bioavailability and altering the kinetics of chemical toxicity. Further analysis will compare the impacts of microplastics on the toxicity of dletamethrin and dimethoate, to understand how the presence of microplastics may affect toxicity of pesticides based on differences in binding capacity. We hypothesise that the toxicity of deltamethrin will be more significantly influenced by the presence of microplastics due it its high hydrophobicity.

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Chronic effects of polystyrene microplastics on freshwater benthic macroinvertebrates
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Understanding the interaction between microplastic and biota in freshwater systems has been identified as a high priority research need. The study of the impact of microplastics on aquatic organisms has mostly been focused on microplastic ingestion and knowledge on the ecotoxicological effects is still limited. The majority of the species studied are pelagic, while sediments have been recognized as an important sink for microplastics . This implies that especially benthic communities may be at risk. In fact, it has been found that the growth and reproduction of the amphipod *Hyalella azteca* decreased after a chronic exposure to polyethylene microplastics. Also, the uptake of polymethyl metacrylat by the amphipod *Gammarus pulex* and the worm *Lumbriculus variegatus* has been reported. This suggests that benthic macroinvertebrates are likely to be affected by the uptake of microplastics. Furthermore, the susceptibility to such effects is likely to be highly dependent on species specific traits. In this study, we determined the effects of polystyrene microplastics on the survival and growth of five freshwater benthic macroinvertebrates with different living and feeding strategies: the amphipod *Gammarus pulex*, the isopod *Asellus aquaticus*, the bivalve *Sphaerium corneum*, and the worms *Lumbriculus variegatus* and *Tubifex spp*. For this purpose, chronic single species toxicity tests were performed in quadruplicate using seven concentrations of polystyrene microplastics with a diameter ranging from 20 to 315 μm. The concentrations tested range from 0 to 40% (g plastic/kg sediment dw) and include the highest microplastic concentration found in a freshwater sediment (1 g

plastic/kg sediment dw). Exposure to polystyrene microplastic concentrations caused no effects on the survival of *G. pulex, A. aquaticus, S. corneum* and *Tubifex spp*. and no effects were found on the reproduction of *L. variegatus.* No significant differences in growth were found for *A. aquaticus, S. corneum, L. variegatus* and *Tubifex spp.* at any microplastic concentration.In contrast, individuals of *G. pulex* exposed to sediment containing high microplastic concentrations (from 10 to 40%)showed a significant reduction in size compared to the controls. These results indicate that the risk of environmentally realistic concentrations of microplastics is negligible, but shows that plastic particles can have a species specific effect on aquatic organisms.

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Pollutants sorbed on environmental sample of microplastics are truly toxic to fish cell lines and Japanese medaka embryos and larvae
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Amount of plastic debris in marine ecosystem is steadily increasing. Microplastics are tiny plastic fragments with diameters < 5mm. They result from runoff and weathering breakdown of larger plastic debris or from cosmetic, chemical industry and domestic sources. The accumulation of microplastic particles (MP) in aquatic environment represent an emerging concern. Indeed, these small particles could be ingested directly by organisms and cause chronic physical and/or toxicological effects. MP are the support for many pollutants present in aquatic environment, especially hydrophobic substances. Although numerous studies have documented the MP uptake and retention in various organisms, impacts on aquatic organisms are little studied so far. With this aim in view, rainbow trout liver cell line RTLW-1 was used to evaluate the toxic effects of organic extracts of MP artificially coated with BaP and environmental samples of MP with different assays (MTT, EROD, comet assay). In addition, *in vivo* studies were conducted with the early life stages (ELS) of Japanese medaka exposed to organic extracts of MP or directly to MP in suspension in water. Environmental samples of MP were collected during the Race for Water Odyssey on beaches of marine islands located in MP accumulation area including Hawaii, Bermuda, Guam, Azores, Easter island. No toxicity was observed for virgin microplastics whatever the bioassay used. Cell line and medaka ELS exposed to MP organic extracts from BaP coated particles showed significant EROD and DNA damage induction. For environmental samples of MP, different patterns of contamination were observed in cell line, probably linked with environmental conditions or polymer composition. Dose dependent effects were observed on embryos exposed to the organic extract of Hawaii sample on EROD activity induction, biometry and fish reaction to light stimulation. Reduced impacts were shown on medaka larvae following trophic exposure probably due to low plastic consumption. Both *in vivo* and *in vitro* tests proved to be sensitive tools to evaluate the potential chemical toxicity of MP. At least one MP from the field was shown to be toxic both on fish cell line and fish embryos.

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Nano-sized polystyrene impact on individual and embryo of fish and trophic transfer in freshwater food chain
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In these days, microplastics are the major pollutants in marine and freshwater ecosystems as enormous amounts of plastic wastes are produced and discarded. These plastic wastes can be eroded and weathered into small plastics, and these small plastics of < 5 mm are called microplastics. They harm the ecosystems and organisms in the ecosystems. These microplastics can be weathered and broken into smaller plastic particles that called nanoplastics (< 100 nm). Generally they have a large surface area and distinctive characteristics. In this study, we carried out the individual toxicity tests on four ecospecies (*Chlamydomonas reinhardtii*, *Daphnia magna*, *Oryzias sinensis* and *Zacco temminckii*) in freshwater ecosystems and trophic transfer test on these species using green fluorescent nanoplastics. First, we exposed nanoplastics to each test species and investigated the individual toxicity and the embryo toxicity of *O. sinensis*. We investigated the growth inhibition (*C. reinhardtii*), mortality and abnormality (*D. magna*), locomotive activity and embryo toxicity (*O. sinensis*) and liver morphology, total cholesterol in blood and locomotive activity (*Z. temminckii*). Secondly, we conducted the trophic transfer test using these four test species and visually analyzed the transfer of nanoplastics. As a result, there was no significant adverse effect of nanoplastics on the growth inhibition of *C. reinhardtii* and mortality and abnormality of *D. magna*. However, there were significant effects on the locomotive activity, embryo, liver morphology and total cholesterol of fish. Fish swim the limited areas when they were exposed to nanoplastics and there were abnormality in the liver of fish exposed to plastic. The level of total cholesterol increased and embryo toxicities were observed. In the results of trophic transfer test, the trophic transfer of nanoplastics was visually confirmed in the gastrointestinal tract and feces of test species. In conclusion, nano-sized plastics can be transferred through the food chain and they can cause the adverse effects on the organisms living in freshwater ecosystems. *This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2016R1A2B3010445).*

Risk assessment and remediation of mine sites and processing sites

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Impact of landfill on the quality of mountain river water
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Waste landfill is a problematic legacy for metal manufacturing industries due to their potential impacts on the environment. Environmental regulations require from metallurgy companies to regularly monitor metal concentrations in the different aquatic compartments surrounding landfills, to compare them with established quality criteria and to conclude on their possible impacts. However, such approach remains limited due to the known importance of metal speciation and overall water quality parameters on metal bioavailability. The use of the Biotic Ligand Model (BLM) may address this gap with the prediction of metal speciation and effects on aquatic organisms. The goal of our study is to compare the BLM prediction with the direct measurements of metal accumulation and effects on various organisms in a study case to evaluate its usefulness to assess the water quality of potentially impacted systems. The studied sites are two mountain rivers (the Isère River and the Doron de Bozel River) of the French Alps, which have landfills along their course. Water, sediment, bryophytes, biofilms and fish (*Salmo trutta fario*) are collected and analysed for their metal concentrations. Water is also analysed for its physico-chemical parameters, DOC, major ion concentrations and sediments for their composition. Biofilms are further examined for their diatom composition and frustule deformation. Finally, bioassays are performed on water (with algae and daphnia) and sediment (with ostracods and plants). Landfill did not impact the pH, conductivity and DOC concentrations of both rivers. However, a 2-fold increase of [Cl] and [Na] was observed in both rivers downstream non-rehabilitated landfills. Increase of dissolved [As], [Co] and [Ni] in the Isère River was further measured. Water quality modification of the Doron de Bozel River was more challenging to evaluate due to high metal concentrations in one of its affluent. A discrepancy was found between metal concentrations in bryophites, but not in fish muscles, and dissolved metal concentrations. *Daphnia magna* were not affected when exposed to river waters. In contrast, 32 % and 45 % of ostracod mortality was found in the most impacted sediment in the Isère River and the Doron de Bozel River, respectively. A water quality degradation of both rivers was thus observed but the landfill involvement remained uncertain. BLM modelling will be our latest step to relate measured and predicted landfill effects on aquatic organisms.

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Effect of a major flood event on catchment metal flux in a catchment subject to mine water remediation
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Flash Flooding from Intense Rainfall (FFIR) events are predicted to become more commonplace in western Europe with global climate change. In catchments with the legacy of metal mining, this may have both positive and negative environmental impacts. This study assesses metal concentrations in fluvial sediments and in the water column before and after a major FFIR event in northern England in 2015. Storm Desmond in December 2015 brought with it the most intense rainfall recorded in the UK (>340mm rain in 24h in NW England) and led to widespread flooding and landslides. The study catchment is the Coledale Beck; a catchment subject to historical mining (Pb-Ba-Zn) in headwater areas and has a recently commissioned mine water remediation scheme in place for addressing the major Zn-rich mine discharge. The data collected suggests that the flood event and resultant landslides did not signficantly affect total sediment-bound metal concentrations (e.g. Zn and Pb) in the catchment. However, there are changes in instream metal concentration apparent after the event. Attenuation of dissolved metals under baseflow conditions appears to be enhanced after the flood event. The mine water treatment system performance was not affected by the flood and this increased instream attenuation is most likely ascribed the the influx of uncontaminated hillslope soils and sediments into the stream which are rich in organics and Al/Fe oxyhdroxides. The implications of extreme FFIR events on catchment scale mine water management are discussed.

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Risk assessment and remediation of contamination in legacy mine sites: experiences from Cartagena-La Union (SE Spain)
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The legacy mining site of Cartagena-La Union in SE Spain poses considerable threat to human health, safety and the environment. Large amounts of wastes characterised by high concentration of metals (mainly As, Cd, Pb and Zn) and poor conditions for plant development are spread over an area characterised by low rainfall and high erosion and evapotranspiration rates. The Sustainable Use, Management and Reclamation of Soil and Water Research Group (GARSA) at Universidad Politécnica de Cartagena (Spain) has a long history in dealing with such issues. The approaches comprise the characterisation of the contamination, evaluation of the environmental/human health risk and proposal of remediation solutions. This manuscript introduces to the environmental and human health risk related to the legacy mining district of Cartagena-La Union in SE Spain. Our aim is to provide an overview of the research activity of the GARSA group (past, present and future) to respond to such challenge. We have a long-term activity in the characterisation of tailings ponds in mine sites and assessment of their environmental and health risk. New approaches involve the use of field spectroscopy techniques (i.e. X ray fluorescence and infrared spectroscopy) for better, quicker and cheaper access to tailings ponds characterisation and condition. We have been involved in a number of projects related to the restoration mine and processing sites. One of the focuses has been related to the development of Technosols from mining wastes following the amendment with different by-products. This has comprised both laboratory and real-scale restoration projects, and monitoring the latter. We have recently demonstrated that biochar and marble waste amendments improve mine waste structural stability which results in a strong impact in reclaiming the physical quality of mine tailings. We are currently evaluating plant effect in the evolution of properties of those Technosols and metal retention (phytostabilisation). In summary, this manuscript shows results of studies that have successfully helped to solve or improve our understanding about complex issues observed in the mining district of Cartagena-La Union.

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When it has to be done again... challenges in the management of a decade old "remediated" legacy site
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A minerals processing site in Marignac, South-West of France operated for almost a century (1916-2002) a wide range of industrial products – mainly magnesium – with waste materials deposited onsite. In 2005, this 16ha site was remediated with two former industrial platforms and three landfills. Shortly thereafter liabilities were transferred to a new company with a poor data transfer. However, within a decade, it became apparent that one landfill was still chemically active causing gas emissions, fissures and potential slope instability. On-going reactivity at the site triggered a suite of environmental investigations (gas emissions, mineralogy, and leaching behaviour) to obtain a better understanding of chemical reactions and the risk associated for human and local ecosystems. Both acetylene and ammonia were measured in atmospheric emissions while high concentrations of ammonia, hydrogen cyanide and dihydrogen in the working atmosphere were measured during coring (NH_3>500 ppm, HCN>30 ppm, H_2>1,500 ppm). In regards of mineralogy, substantial heterogeneity was apparent both verticality and horizontality, likely related to different wastes deposited and internal reactions that occurred over past decades within the landfill. Eight major groups can be identified from analysis of the mineralogical data with a further four major geochemical/mineralogical associations found. The ratio of Cao/MgO was found to be an important proxy variable that could define geochemical reactivity within the pile. If CaO/MgO was < 5 then a strong likelihood existed for higher temperatures and gaseous (NH_3, VOC, and HCN) emissions and intermediate to high pH (ca. 9.5 – 12.5). On this basis it may be possible to subdivide waste to target intervention. Layered double hydroxides (e.g. hydrotalcite) are found to be important passivating secondary minerals at the ambient pH (ca 9.5 – 12.5) in waste materials. Formation of additional LDH minerals is probably limited by supply of Al. Based on a characterisation of waste materials present, preliminary conclusions were drawn with respect to a potentially successful rehabilitation at this landfill of concern. It is suggested that in the first instance, a cover be emplaced on the waste material to restrict further water ingress. In addition, active venting, monitoring and treatment of gaseous emissions be undertaken to reduce the local environmental impact.

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Evaluating the success of different rehabilitation strategies of bauxite residues: a risk assessment of trace elements along the food chain
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Bauxite residues are the by-product of the aluminium industry, resulting from the extraction of alumina from bauxite ore through the Bayer process. To date, approximately 2.7 billion of bauxite residues have been produced, with an increasing global rate of 120 million tonnes per year. Bauxite residues disposal has raised concern worldwide especially following the accident occurred in Ajka (Hungary) in 2010. Due to the presence of trace metals (such as Al, As, Cd, Cr, Ga, V), bauxite residues represent a potential source of pollution for air, soil and water, both on site (in the disposal area) and off-site (in the surrounding areas). Nowadays, phytostabilization (i.e. revegetation) has been proved to be a promising, cost-effective and non-resource intensive option for bauxite residues rehabilitation. Its primary objective is to stabilise residues against air dispersion and water erosion but, at long-term, a successful revegetation should also aim at creating a sustainable soil ecological community, capable of performing essential soil functions. So far, a number of strategies (e.g. amendments, capping, neutralisation, etc.) have been successfully tested so as to overcome the inherent physicochemical characteristics of the bauxite residues (mainly high salinity, high sodicity, high alkalinity) that hinder plants growth. However, there are still few studies evaluating the long-term success of the rehabilitation programme and most of them overlook important ecotoxicological aspects. The present study aims to assess rehabilitation strategies for bauxite residues considering the ecotoxicological risk to those organisms that live or feed in the rehabilitated areas. To this end, samples of bauxite residues/soil, plants and animals were collected from different sites so to be representative of various rehabilitation strategies and histories. Selected physicochemical parameters were characterised in the bauxite residues. Content of trace elements (both essential and non-essential) in residues/soils, plants and animals were determined by ICP-MS. Finally, ecotoxicological bioassays (such as Phytotoxkit and Rhizotest) with plants and biomarkers analysis with macroinvertebrate were conducted. In conclusion, through a multidisciplinary approach, the present research is likely to provide useful guidelines for the selection of future effective rehabilitation programmes for bauxite residues

The challenges of Life Cycle Sustainability Assessment (LCSA) of energy technologies

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Sustainability impacts of energy system scenarios in Spain
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Several decarbonization policies have been put in place in Europe in the last years with the ultimate objective of combating climate change and reducing energy dependence. All of these energy transition policies aim to achieve a more competitive, secure and sustainable energy system but mainly focus on aspects of reducing global warming emissions, increasing renewable energies penetration and increasing energy efficiency. However, other environmental impacts and socioeconomic benefits are not always taken into account. The aim of this study is to perform a sustainability assessment of various climate and energy scenarios up to 2030 in Spain. This study will estimate the socio-economic benefits, in terms of value added and job creation, and CO2 emissions and other environmental impacts associated to different electricity scenarios. Input-Output Tables and social accounts from the World Input-Output Database project have been used to create a multiregional analysis. The evolution of the Spanish electricity system in the long term through different scenarios is the result of using the energy optimization model TIMES-Spain. The model will provide information concerning the investment and operation costs from the different technologies of the electricity system of each scenario. Results will show direct and indirect environmental and socio-economic impacts by technology, distinguishing between investment and operation and maintenance for each scenario and identifying those world regions and sectors that are most economically stimulated and those that are the main contributors to environmental impacts.

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Life Cycle Sustainability Assessment for photovoltaic panels using soca
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"Ensure access to affordable, reliable, sustainable and modern energy for all" is one of the UN Sustainable Development Goals (SDG) defined in 2016. To achieve this target, not only current and future ecological aspects should be considered, but also social and economic elements regarding entire life cycles should be addressed. This is still rather difficult because no methodological structure for Life Cycle Sustainability Assessment (LCSA) was agreed on. Furthermore, databases or other tools allowing the calculation of environmental, economic and social impacts of product life cycles at a time do not exist yet. Therefore, an add-on for the ecoinvent v.3.3 database, called soca, was developed containing social impact information. The aim is to combine Social and Environmental Life Cycle Assessment (S-LCA, E-LCA) and Life Cycle Costing (LCC) in one single database. To add social indicators to ecoinvent, the generic social inventory information of PSILCA was mapped to all processes in the ecoinvent database, with exception of market processes and activities used for database administration and modelling. The information is provided as risk-assessed indicators modelled as elementary output flows for every activity in ecoinvent. For every process, the required working time to produce the reference product was calculated to measure and quantify the risks of each social indicator. Data quality assessment complements the information and documentation on the process level. As a result, soca covers social impacts on workers, local communities, societies and value chain actors. It contains more than 50 social indicators related to several subcategories. The combined calculation of environmental, economic and social life cycle assessment was tested on a small case study comparing the production of photovoltaic electricity in Germany, India and Mexico. The preliminary results showed the importance of combining the results of the three areas of sustainability in the decision-making. Added to

ecoinvent v.3.3, soca completes the environmental and cost information already existing in the database by social aspects. This results in the first complete foundation for Life Cycle Sustainability Assessment that enables the user to combine E- and S-LCA and LCC using one single database and, hence, compare impacts for exactly the same product system. It is a promising advance for LCSA and a starting point for further developments in the field.

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Life Cycle Sustainability Analysis of an innovative configuration of Concentrated Solar Power technology
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This work responds to the need expressed by the scientific community to test LCSA methodology in different products and sectors. The methodology employed in this work is based on the *LCSA Analysis* approach and has been applied to evaluate the sustainability (including environmental, economic and social dimensions) of a Concentrated Solar Power (CSP) plant based on HYSOl technology, an innovative configuration that delivers improved efficiency and power dispatchability. The main goal of the study is to answer the following two questions: 1) "to what extent may the HYSOL technology improve the sustainability of power generation in the Spanish electricity sector?", and 2) "In the context of a future Spanish renewable energy plan, which CSP technology should be favoured considering sustainability criteria?" To this purpose, a number of sustainability sub-questions were posed which helped to choose the appropriate tools required to produce the adequate results and answers. In the environmental area, attributional and consequential LCAs were performed in order to determine and compare the potential environmental impact of the HYSOL technology in two alternatives (HYSOL BIO for operation with biomethane and HYSOL NG for operation with natural gas). In the economic area, a Life Cycle Cost Analysis and a Multiregional Input Output Analysis were performed in order to estimate the life cycle costs and economic effects of the HYSOL BIO, HYSOL GN and conventional CSP plants. In the social area, a Social Life Cycle Assessment was applied following UNEP/SETAC guidelines and a social risk assessment of was performed using the Social Hotspots Database. The results obtained in these three areas were integrated using a "questions and answers" layout, each one describing a specific element of sustainability. Visual diagrams representing the sustainability of the analysed scenarios were also produced in order to facilitate the interpretation of results and decision making while answering the second question of the analysis. The analysis suggest that the HYSOL technology operating with biomethane exhibited improved environmental and social sustainability than with natural gas, although the economic sustainability of the former is lower than the latter. The results also indicated that the technology innovation developed under the HYSOL project is well aimed to improve the sustainability of CSP technology and the Spanish electricity sector.

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Simplifying the integration of energy system models and LCA
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Energy system models are increasingly used in junction with life cycle assessment (LCA) to inform energy policies of possible burden-shifting and co-benefits of interventions. A particular strain of models that has gained momentum in the integration of LCA and energy systems are those based on the TIMES model generator. However, the integration between the two models presents considerable challenges. A typical TIMES model contains thousands of processes, therefore, the mapping (i.e. finding the equivalent processes between the TIMES and an LCI database) can be extremely time-consuming. Here we propose a transparent open-source soft-linking framework to integrate data of TIMES-models into LCIs, including a screening algorithm to identifies the most relevant processes based on their contribution to changes in global warming emissions, limiting the number of processes that need to be mapped. The framework is applied to assess the effects of household risk-aversion on the plans to meet CC mitigation targets of the province of Quebec (Canada), using the North American TIMES Energy Model (NATEM). The LCA impact assessment is done using Impact2002+ endpoint using the LCA software Brightway 2. A baseline and alternative scenarios are modelled, and differences between both scenarios are used to construct the inventory, following the consequential LCA paradigm. Results indicate that when we just consider the processes that contribute to 99% of the total change in GW emissions between scenarios, instead of all the process that change emissions, we reduce by sixfold the number of processes that need an LCI equivalent. Therefore the screening process vastly simplifies the integration of models. The marginal electricity production as predicted by NATEM is compared with the existing consequential model of ecoinvent and results suggest impact may be higher than expected, particularly in non-GW categories. When aggregated by sector, results indicate that the risk aversion introduced in the residential sector rises GW emissions and these needs to be compensated with higher reductions in the transport and commercial sector. This illustrates potential interactions between sectors, that have been rarely assessed in the LCA literature.

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Combining LCA and a prospective techno-economic energy model to assess the sustainability of emerging energy pathways: the case of integrated forest biorefineries
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In the current context of the fight against climate change, the energy system will experience transformative changes in the coming decades. The life cycle assessment (LCA) methodology is increasingly used to assess potential environmental impacts of emerging low-carbon energy pathways. However, other issues may arise when assessing their sustainability. Indeed, their large-scale deployment may lead to indirect impacts due to changes elsewhere in the energy system. Moreover, their economic viability may depend on several factors such as climate policy or technological innovations, that are not taken into account in traditional techno-economic assessments performed at the process design stage. The complexity of these interrelated dimensions requires the use of a holistic approach combining several methodologies. In this paper, we propose an approach combining the LCA methodology and a TIMES energy system model to assess potential environmental impacts and market penetration of an emerging energy pathway under different prospective climate policy scenarios. We apply this approach to the integrated production of butanol in a Canadian Kraft dissolving pulp mill. A simulation of this process has been developed with the Aspen Plus software to provide input data (material and energy flows) for both the LCA and the TIMES models. Optimization runs are then carried out using the NATEM-Canada (TIMES) model for three prospective scenarios (one reference case and two climate policy cases). Finally, potential indirect impacts associated with the deployment of the butanol production process are assessed using LCA and comparing how the final energy demand is met for a given climate policy scenario with and without access to the butanol production process. The results show that the level of energy integration at the process implementation stage is crucial to make butanol from integrated forest biorefinery a preferred alternative to traditional fuels to reduce greenhouse gas emissions, and to ensure its economic competitiveness. Hot spots are identified to help researchers and engineers designing a more sustainable butanol production process. Sensitive variables and conditions required to ensure better environmental performance and market penetration are also identified. This study offers a general framework to perform prospective assessments of other emerging energy pathways.

Regulatory Best Practices for Assessment of Endocrine Active Substances

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Comparing existing regulatory frameworks for endocrine disrupting chemicals across the globe
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During the last decades, the regulatory communities in different parts of the world have been debating how EDC should be specifically addressed in a regulatory context. Due to the current scientific complexity and high uncertainty in relation to identifying and characterizing EDCs, regulatory approaches specific to EDCs are still scarce. As a part of an initiative by the International Panel on Chemical Pollution (IPCP) to synthesise existing knowledge on EDCs, we have examined regulatory frameworks specifically addressing EDCs across countries. In particular, we aimed at providing an overview of, and identifying potential gaps in, existing regulatory frameworks for EDCs. Our analysis shows that existing frameworks and efforts show a high degree of variation: while some regulatory approaches cover general chemical groups, such as "pesticide chemicals", others focus on specific substances in specific applications, such as bisphenol-A in food packaging materials. Furthermore, the existing frameworks and legislations span over a wide range of regulatory domains, such as water pollution, consumer products or food packaging materials. This creates additional complications in regulatory efforts within a jurisdiction. In particular, the executive responsibilities over these domains are often held by different authorities within the jurisdiction. Thus, efficient and effective communication between these institutions is crucial for a consistent regulatory approach with regard to EDCs within the jurisdiction. However, this may not always be the case, as our analysis indicates that inconsistent definitions and languages are used in different regulatory frameworks. Our current and ongoing in-depth analysis, including understanding existing efforts in developing and transition countries, may serve as a basis for future discussion on harmonizing efforts across jurisdictions.

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Considerations for Developing, Validating and Implementing Performance Based Test Guidelines
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ER2
To manage ongoing development of test guidelines that evaluate the same endpoint, OECD developed guidance on a new concept known as Performance Based Test Guidelines (PBTG). The PBTG concept creates a grouping of test methods, with similar essential components and endpoints, to ensure that the methods have equivalent performance. This concept is not entirely foreign to ecotoxicologists, where many guidelines have historically included the option of testing any one of a number of species provided they adhered to specific performance standards. However, it is a relatively new concept for in vitro assays and it is being implemented to manage the proliferation of *in vitro* test systems. In addition, EPA is interested in adopting performance based standards to manage acceptance criteria for high-through-put *in vitro* screening assays that will inform the EDSP. An overview and analysis will be drawn from the two initial PBTGs developed by OECD, the estrogen receptor transcriptional activation assays and the *in vitro* skin corrosion assay. This presentation will provide an overview of the general approach to implement PBTGs and standards, examples of assay types where PBTGs have been applied, approaches and considerations to cross-validate mechanistically and functionally equivalent "me too" assays, recommendations, learning's from these first two efforts, and a discussion of advantages and disadvantages of implementing PBTGs.

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Applying high-throughput screening data to define early key events for impaired vitellogenesis
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Recent advances in molecular biology and computational toxicology have enabled high-throughput screening (HTS) for large chemical inventories across a wide range of toxicity pathways. Within the adverse outcome pathway (AOP) framework, this information is useful to define early key events that lead to apical effects of regulatory concern. For example, several endocrine modes of action (i.e., androgen receptor agonism, estrogen receptor antagonism, aromatase inhibition) are linked to reduced synthesis of vitellogenin, the egg-yolk precursor protein, which leads to impaired reproduction in fish. However, non-endocrine mediated pathways, such as aryl hydrocarbon receptor agonism, mitochondrial dysfunction, and oxidative stress, can also impair the production of vitellogenin. These alternative mechanisms confound interpretations of the Fish Short-Term Reproduction Assay (FSTRA), which is used to identify endocrine-active substances in the USEPA Endocrine Disruptor Screening Program (EDSP). Thus, the objective of this study was to leverage HTS data to define early key events for endocrine and non-endocrine toxicity pathways leading to impaired vitellogenesis. AC50 values were extracted and normalized for 10 ToxCast assays related to endocrine and non-endocrine modes of action. Then, compounds tested in all assays (5592 total) were used to develop ToxPi figures, where the size of each slice was proportional to the potency for selected a mode of action. A comprehensive review of data evaluation records from the EDSP was performed to identify compounds (10 total) that decreased plasma vitellogenin levels in the FSTRA. ToxCast data were available for 9 of these substances, and there were 5 compounds (Z-Tetrachlorvinphos, Propiconazole, 2-Phenylphenol, Flutolanil, Metolachlor) where non-endocrine toxicity pathways were active at lower concentrations than endocrine toxicity pathways. Finally, a principal component analysis determined these compounds had similar HTS profiles to model compounds for aryl hydrocarbon agonism, mitochondrial dysfunction, and oxidative stress. These results suggest non-endocrine toxicity pathways are likely involved in the AOP for impaired vitellogenesis, and there remains a research need to further define key events for these alternative pathways.

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Medaka (Oryzias latipes) Multi-Generation Test with Triclosan; An Additional Piece of the Weight of Evidence Assessment Puzzle
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Triclosan (TCS) is an antimicrobial agent used in a wide range of personal care and consumer products, such as deodorants, cosmetics, dental care, clothing and household items. As with most personal care products, TCS can be rinsed down the drain and transported to wastewater treatment plants. While TCS is biodegraded, and can sorb to biosolids, some TCS enters the aquatic environment and may expose ecological receptors. Given the potential for exposure to both humans and wildlife, resolving whether or not TCS should be considered a potentially endocrine active compound is important given increasing worldwide concern about endocrine disrupting substances and their potential for causing adverse effects on public health and the environment. In order to add data to the hypothesis-driven weight-of-evidence evaluation of potential endocrine activity of TCS, three generations of Japanese medaka (*Oryzias latipes*) were exposed to nominal concentrations of 1.4, 2.8, 5.6, 11 and 23 μg/L TCS and a dilution water control. The study started with exposure of young adult fish in reproductive condition (the F0 generation). Eggs collected from the F0 generation were used to initiate the F1 generation and were subject to maternal transfer and gamete exposure of the test substances if it were to occur. These eggs were then monitored through sexual development (82 days) in the F1 generation. The exposure continued through reproduction in the F1 generation and was continued through hatch of the F2 generation. There were no treatment related effects on survival, growth, or reproduction in the F0 or F1 fish, with the exception of a reduction in growth in F1 male fish in the highest test concentration (23 μg/L triclosan). Slight reductions in male anal fin papillae counts at the 23 μg /L level were consistent with apparent growth effects at this level and were attributed to general toxicity and not an endocrine mediated effect. In the high treatment group, histological evaluation of liver and kidney tissues showed effects consistent with general toxicity supporting the conclusion that effects on weight and anal fin papillae were not endocrine mediated. The results of this study are in agreement with the other lines of evidence available in the hypothesis-driven weight of evidence evaluation of triclosan, supporting the conclusion that triclosan is not acting as an agonist or antagonist within the estrogen, androgen, thyroid, or steroidogenic pathways.

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Propiconazole: a case study for the practical implementation of the EU Commission ED criteria
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In June 2016 the European Commission released the first draft of its criteria for the determination of endocrine disrupting properties. The definition utilised is the WHO/IPCS definition and, in the context of the criteria for protection of the environment, requires an endocrine mechanism, a population relevant adverse effect and a biologically plausible causal link between the two. Although the population relevance of effects is often assumed, adverse effects such as reproductive impairment and sex ratio changes, are commonly recorded within routine laboratory toxicity studies. In addition, both in vitro and in vivo endocrine relevant mechanistic endpoints are becoming more commonplace. However, what is often given less attention, is the causal relationship between the proposed mechanism and the adverse effect. There are often multiple complex indications from numerous different potential mechanisms of toxicity; therefore, a detailed weight of evidence evaluation is required to ensure correct identification of chemicals of concern. Data will be presented, as part of a weight of evidence, from in vitro and in vivo screening studies, along with higher tier studies, conducted with the fungicide propiconazole. The weight of evidence analysis will focus on data from a series of fish studies examining multiple endpoints relevant to the endocrine system and other toxic modes of action. Discussion of data will focus on the evidence for endocrine mechanisms in vivo and the potential impact of systemic toxicity, particularly hepatotoxicity, in the context of causation of adverse effects. For propiconazole, the relevance of potential endocrine versus non-endocrine mechanisms for the observed adverse effects will be demonstrated. The weight of evidence presented leads to a conclusion that, based on the comprehensive dataset already available, propiconazole does not fit the definition of an endocrine disruptor according to a reasonable interpretation of the EU Commission criteria.

Clean circular economy: recycling while eliminating legacy toxics

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Rubber granulates: proper recycling or toxic waste?
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Recycling polymeric materials addresses the need for resource efficiency in a finite world, but at the same time it raises questions about toxic compound residues being introduced into new products made from recyclates1. This study investigates the toxic residues in recycled tire rubber granulates on artificial-turf soccer fields. In the Netherlands a discussion has started regarding the exposure of soccer players to carcinogenic residues in recycled tire rubber granulates when playing on artificial-turf soccer field. Some soccer clubs have already replaced the field and other clubs have even forbidden to use the field as long as no clear answers could be given regarding the health risks. In total 120 ton of rubber granulate is used on one artificial-turf soccer field and every year another 300 kg is added to maintain the field. On one hand the recycling of rubber tires for artificial-turf soccer field reduces the overall burden of rubber waste on landfills but on the other hand by using this rubber granulates on artificial-turf soccer fields it still ends up into the environment. Also during rainfall toxic residues can easily leach from the granulates into the environment. Therefore, this study also investigated the he toxicity of 7-days leachates of the rubber granulates on zebrafish embryos. Seventeen PAHs were detected in the rubber granulates collected from the eight soccer fields. The ?PAHs varied from 18 to 40 mg/kg. Pyrene was the dominating PAH with levels up to 18 mg/kg. Also the ? carcinogenic PAHs are calculated, which are based on four PAHs which are also included in the 8 EU-PAHs for which restriction have been set by REACH. The ?4 PAHs (2.1-5.3 mg/kg) in the rubber granulates of the eight soccer fields all exceed the REACH limit of 1 mg/kg. At the highest exposure concentration (100% leachate) zebrafish larvae showed a significant reduction in hatching. This caused an increased mortality in fish older than 4 days. Fish exposed to 100 % leachate showed a strong cyp1a induction in the

liver. The PAH levels in the recycled tires rubber granulate exceed the EU-REACH limits given for PAHs in consumer products. For the 7-days leachates increased mortality was observed in zebrafish embryos. More research, for example screening for other toxic substances, exposure routes, other toxicity parameters, is recommended to further investigate the health risk of recycled tire rubber granulates.

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Ensuring chemical safety of food contact materials and articles in the circular economy

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Food contact materials and articles are essential to the production, storage, handling, transport and preservation of foodstuffs. Food contact materials (FCMs) like plastics, printing inks or coatings are combined to make food contact articles (FCAs), like conveyor belts for food processing or cans for food packaging. In a circular economy, the presence of chemicals of concern (COCs) in food packaging has implications for the current approach to risk assessment, where chemical exposures are regulated: Reusing and recycling FCAs may lead to unforeseen and, therefore, unmanaged exposures to COCs. This is because not all future applications of a material are predictable in the circular economy, with closed material cycles. Therefore, the functioning of a sustainable, circular economy depends on materials being free from COCs from the onset of production and remaining so over their entire life time. Especially food packaging has a major impact on sustainable food consumption as it is essential for preserving foods. Preventing food waste is of societal interest since the environmental impact of food production is large. However, packaging-related plastic waste also has an environmental impact, and understanding its role for human and environmental exposure to COCs is important. We have shown that 175 known COCs can be legally used in FCMs, and some of these substances migrate into foods. Therefore, FCAs as a relevant exposure source for COCs needs to be taken into consideration. As a consequence, the selection of food packaging materials requires an integrative evaluation so as to not jeopardize the overall goal of an environmentally friendly economy. To achieve this the LCA method could be amended so that all chemical exposures are considered. Here we discuss the current approach to risk assessment of all kinds of FCAs, both virgin and recycled, and demonstrate that it is insufficient for ensuring that food packaging does not contain COCs that may harm human health or the environment. Further, we review recycling processes for different types of food packaging materials with respect to the removal of COCs potentially present. Finally, we suggest a novel approach to establishing the safety of FCAs by employing biological analysis to assess the toxicity of overall migrate from the finished article. We discuss its advantages and limitations, and highlight development needs for making such an approach practically applicable.

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Environmental assessment of presence of impurity materials and chemical pollutants in wood waste meant for recycling

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In the last decades, reuse and recycling of waste have become central to the European Union's environmental policy, and have been promoted as the main driving forces toward a circular economy. Among recyclable materials, wood waste occurs in very large amounts, due to its many applications (constructions, buildings, furniture, packaging) and its bulky volumes. Wood waste is a valuable material as it has potential for both recycling and energy recovery. However, in a circular economy perspective, cascading of material uses should be applied, and wood incineration should be performed only when recycling is not possible. High quality recycling should be preferred, and presence of impurities should be minimized, if not avoided. Impurities in wood waste generally include metals, plastics, textiles, glass, inert, adhesives, paints, waxes, preservatives, fire retardants. The objective of this study is to evaluate the environmental consequences of the presence of impurities in wood waste collected at recycling centers and meant for recyling. For the purpose, 41 samples of pre- and post-consumer wood from different applications were used. The evaluation includes the physicochemical characterization of the samples and the analysis of four different types of impurities: Misplacements: non wooden items. Unavoidable external items: non wooden items that were essential during the use phase of the wood waste. Material detrimental to recycling: wooden items whose properties would lower the quality of a recycled product. Chemical contaminants: elements present in the wood structure whose presence is due to treatment of the wood with chemical products. Results showed that impurities constitute a significant portion of wood waste collected for recycling. The risk of putting chemical pollutants into new cycles was investigated and quantified. Environmental impacts caused by presence of impurities were modelled by life cycle assessment. This study thereby shows that recycling alone may not lead to a clean circular economy and additional efforts are needed for an improved recyclable feedstock. Social awareness, proper sorting of the waste at collection points, preliminary treatment and sorting steps at recycling facilities are needed to enhance the quality of wood waste for its second life in a new product. This research contributes to the general discussion that an additional focus on quality is needed in recycling activities.

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Cleaning the WEEE plastic loop in the Indian informal sector

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The informal plastic recycling sector in India achieves much higher recycling rates than seen in Europe by operating with lower economic costs. It also provides livelihood to many, but is done with little regard for worker safety and environmental protection. Plastics containing hazardous additives like brominated flame retardants (BFR-plastics), whose recycling is severely restricted by international legislation, are nevertheless collected, reprocessed and included in new products in India. This brings concerns over exposure risks for workers and consumers. In this presentation, we report intermediary results of a project aiming to set-up a take-back system in India to "pull" out BFR plastics from Indian recycling systems and establish proper local treatment options. We identified three *sine qua non* conditions for a successful take-back system to be implemented: (i) availability of appropriate methods to identify and sort out BFR plastics in informal settings (technical feasibility), (ii) existence of economic incentives for recyclers to abandon the recycling of BFR plastics (financial feasibility), and (iii) willingness to cooperate between actors involved in such a take-back scheme through aligning their interests (organisational feasibility). Regarding the first condition, a field sampling and testing campaign was performed in Delhi. Pre-selected separation methods considered as applicable in the informal sector were performed on samples and then tested by analytical methods in order to assess the removal effectiveness of the respective separation methods. Results show that simple separation methods such as density separation are both highly effective and readily applicable in the informal plastic recycling sector. In a stakeholder process, a pilot take-back scheme funded by a producer under its EPR obligation by Indian law has been developed. Proposing such a scheme requires considering concerns voiced by all affected parties and finding ways to align their interests. One of the key elements of the scheme is the inclusion of a neutral third party due to trust deficits between stakeholders and the need for partial anonymity. Considering the enormous challenges posed by legacy substances in combination with the stated goal to move towards more circular economies, the presence of informal recycling chains brings additional complexity. However, the presented take-back scheme provides a potentially viable approach to address these issues.

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A sustainable solution for HBCD containing PS foam via a dissolving technique with bromine recovery

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Today for a circular economy, the most preferred option is material recycling as it has the lowest carbon footprint. In Europe packaging waste has the highest recycling rate with more than 50% for certain plastics streams. The flame retardant (FR) hexabromocyclododecane (HBCD) is classified as a SVHC under REACH, listed as a POP under the UNEP Stockholm convention since 2013 and prohibited for recycling. The main use of HBCD is in insulation PolyStyrene (PS) foams. Recyclates with more than 100 ppm HBCD under the EU POP regulation are not allowed to put back on the market. Moreover, all waste containing HBCD and its disposal is restricted as HBCD has to be destroyed. It means that the only way to get rid of HBCD containing material is high temperature incineration, but across Europe we have insufficient incineration capacity. The Plastics Industry did a trial with non-recyclable PS foam streams, e.g. from building and construction market. This has been co-incinerated in a full-scale waste incineration plant. The co-feeding of 1 and 2 wt% of polystyrene foam had no influence on the operation of the plant. The air emission, including that of dioxins and bromine, was not altered and so was the quality of the solid residues. The obtained destruction efficiency for HBCD was >99.999%, again independent of the amount of added PS foam. These clear results indicate a virtually complete destruction of HBCD. However, we strongly believe that incineration should not be the only End of Life option for a material that can easily be re-processed into a raw material without compromising on the quality. In its second life, it would enter the value chain again of which the value chain could benefit. Since 2002 Fraunhofer IVV has been looking into recycling solutions for plastics containing certain flame retardants. As a result, the CreaSolv® Process has been developed as a solution for plastics containing restricted substances, e.g. PS foam containing HBCD, which has been used in large quantities in insulation materials over decades. As a results of these studies a Non–profit foundation under Dutch law "PolyStyrene Loop" aiming to build a demo plant by 2018 for sustainable recycling of End of Life PS containing HBCD including bromine recovery. The objective of this demonstration plant is to prove the technical and economic feasibility of such a facility in being able to close the PS loop via compacting and dissolving the PS foam and separating HBCD. The recycled PS can be re used in the same application whereas the HBCD is destroyed in a hazardous waste incinerator followed by a Bromine Recovery Unit. The valuable bromine is recovered and can be used again to produce a new sustainable and alternative FR: i.e. Polymeric FR for use in PS foams. As it will take time to establish the CreaSolv® Process in Europe, another waste treatment option for PS foam containing the POP HBCD will be used as interim solution: incineration with energy recovery.

Cost effective and ecological relevant testing using invertebrate species: new insights for environmental risk assessment (I)

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Nanomaterial toxicity through a high throughput screening approach on Mytilus edulis hemocytes
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Nano-scale products are being produced faster than current toxicity screening can keep up with, making it difficult to develop a universal regulatory policy. The objectives of the European project NanoReg II, is to provide a means in which nanomaterials can be incorporated into REACH guidelines and to implement safer by design strategies in assessing nanomaterial toxicity. In order to address the potential environmental impact of engineered nanomaterials (ENMs) techniques that can measure large numbers of ENMs in a reliable and cost-effective way need to be used. The use of *in vitro* assays that implement high throughput screening (HTS) strategies can address this growing demand by providing a fast and cost effective means of developing a comprehensive regulatory framework for engineered nanomaterials (ENMs). In this context, the aim of the study was to develop an HTS platform based on toxicity screening through a primary cell culture on *Mytilus edulis* hemocyte cells exposed to different ENMs at different stages of their life-cycle for the implementation of safer-by-design strategies. The current research is focused on testing silicon based ENMs (Si nanomaterials and Si particles coated in carbon) provided by an industrial partner in order to determine the toxicity profiles using the HTS platform. Sublethal concentrations were then selected to measure a battery of biomarker responses implicated in cell viability, apoptosis, oxidative stress, immunotoxicity, genotoxicity. *In vivo* testing was then conducted in order to validate the results. Preliminary results suggest that the size of ENM at production influences toxicity. Results collected *in vitro* will be validated through an *in* vivo exposure.

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Assessing the relevance and reliability of invertebrate embryo larvae studies for risk assessment of pharmaceuticals
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Pharmaceuticals generally have a very specific mode of action and short term acute exposure is not expected to be environmentally relevant. The environmental risks of pharmaceuticals under the current European Medicines Agency (EMA) guidance[1] are therefore assessed based on their chronic risks. For invertebrates and fish this means studies with a chronic exposure duration of at least 21 days. Embryo larvae studies with invertebrates (usually echinoderms or molluscs) are short term tests (48 h) that are often very sensitive because they are targeting effects on the early life stages of the organisms. Standard test methods are available for these species which are mainly aimed at whole effluent testing (WET). Due to the short duration of the test the result from an invertebrate embryo larvae study is frequently the only publically available “chronic” invertebrate endpoint for a pharmaceutical particularly for the marine compartment. In this study the available data from embryo larval tests for pharmaceuticals have been reviewed and it is found that they often show high toxicity in these tests even when compared with full life cycle studies in other organisms that have been designed to target the specific mode of action of the substance. The reliability and relevance of data from embryo larvae tests for the risk assessment of pharmaceuticals are discussed as well as some of the basic assumptions that are made when applying assessment tools such as the criteria for reporting and evaluating ecotoxicological data (CRED)[2] to these data. Embryo larval tests have a valuable role in improving our understanding of the effects of pharmaceuticals in the environment but the use of these tests for limit setting and risk assessment purposes would be improved if the range for effects were better defined, there was better understanding of the mode of action for toxicity and if there was evidence for repeatability in the results. [1] EMA. 2006. Guideline on the environmental risk assessment of medicinal products for human use. Committee for Medicinal Products for Human Use (CHMP), European Medicines Agency (EMEA), London, UK. EMEA/CHMP/SWP/4447/00. 1 June 2006. [2] Moermond CTA, Kase R, Korkaric M, Agerstrand M. 2015. CRED: criteria for reporting and evaluating ecotoxicity data. Environ. Toxicol. Chem. 35:1297-1309

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Assessing effects of the Bt toxin Cry1Ab on trichopterans with a food-spiking method
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The environmental risk assessment of GMO (including Bt crops) concentrated on terrestrial habitats. Only recently, aquatic habitats came into focus. Bt crops produce Cry or Vip proteins leading to the death of target organisms, such as Lepidoptera (e.g. corn borer species). Trichopterans have a phylogenetically close relationship to lepidopterans, which is why they might be sensitive to lepidopteran specific Cry toxins. In the case that aquatic test organisms were exposed to GMO through GM plant material diet, it is difficult to prepare different test concentrations, needed to calculate a dose-response. In this study we investigated the sensitivity of trichopterans to a Cry1Ab toxin using a food-spiking method. Black alder (*Alnus glutinosa*) leave discs were spiked with Cry1Ab toxin by applying solutions with different Cry1Ab toxin concentrations. For investigating the effects of Cry1Ab, studies with leaf-shredding caddisfly larvae (*Sericostoma spec*., *Chaetopteryx spec)* were conducted. The larvae were fed with spiked leave discs in different concentrations over 6 or 12 weeks (10 repl.). The test vessels contained sediment and were placed in a climate chamber at 16 °C. The following endpoints were monitored: mortality, growth, consumption, lipid content and larval development. Furthermore the target organism *Ostrinia nubilalis* was fed with spiked plant material over 7 days in a bioasssay and mortality was recorded in order to verify the food-spiking method. The Cry1Ab toxin concentrations of the spiked leave discs were measured using an enzyme-linked immunosorbent assay (ELISA)(Agdia, Elkhart, USA) and revealed that the spiking method is able to prepare different toxin concentrations. The *Chaetopteryx spec.* larvae showed a slightly higher mortality than the control. The larvae from the control and the treatments showed the same larval development. The *Sericostoma spec* larvae showed no higher mortality than the control. After 6 weeks two treatments revealed a significant slower larval development than the control larvae. *O. nubilalis* larvae showed a high mortality, when exposed to plant material spiked with Cry1Ab toxin. This demonstrates that the spiking method principally worked. The new developed spiking method is a useful method to produce different concentration of Cry toxins on plant material. The results of the caddisfly experiments showed that Cry1Ab toxin might have slight influence if exposure happens as part of their food.

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Development of a testing method simulating running waters for chronic testing of invertebrate species
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Invertebrate species compass a various number of organisms which are already used in ecotoxicological tests including amphipods, choronomids, cladocerans or gastropods. However, the largest group within the invertebrate species are insects. Common laboratory toxicity testing is conducted with a relatively small number of model organisms which were often chosen because of their simplicity in culturing and testing. There are still several stream-dwelling invertebrate species including mayflies and stoneflies which can be more sensitive than standard test organisms. Because of their habitat adaptions testing of those organisms is more difficult than testing of organisms living in standing water bodies. Mayflies and stoneflies are hemimetabolic insects with a complex life cycle divided in a larval aquatic phase and a short adult phase as a flying insect. They occur in cold, fast moving streams which provide a high oxygen level. For our study we used field collected larvae which were adapted to laboratory conditions before test start. The main goal was to develop a testing method for chronic testing of mayfly and stonefly larvae under stream conditions. The biggest challenge was to design a test system which is easy to handle under laboratory conditions but at the same time mimics the natural habitat conditions of the larvae regarding flow, temperature, oxygen level, light intensity and food supply. The developed test system provides a novel approach for the chronic testing of invertebrates particularly with regard to provide more organisms for a species sensitivity distribution. A pilot study with the mayfly *Ecdyonurus venosus* and the stonefly *Protonemura ssp.* exposed to the neonicotinoid imidacloprid was perfomed in November 2016. In contrast to usual indoor stream systems not the water body itself but vessels insight the test containers were moved and circulated through each treatment to obtain the aimed flow. The test design consists of six treatments with ten replicates. As food source small filters were prepared with algae food which could be grazed by the larvae. Black glass nuggets were added to simulate the natural habitat. As endpoints total length, head width and length of wing pads were measured at test start and end. During the test mortality was recorded. The obtained effects of imidacloprid on the tested larvae as well as the test approach will be presented.

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Chronic exposure to field-relevant fungicide concentrations impairs reproduction in the amphipod leaf-shredder Hyalella azteca
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Leaf-shredding amphipods can contribute substantially to the breakdown of leaf litter, an essential process in many aquatic ecosystems. Although the fitness of these shredders as well as the functions they provide may be impaired by exposure to fungicides, a group of substances interacting with highly conserved sites of toxic action, little is known about their effects on shredders' reproductive performance. Therefore, a semi-static 56-day partial life-cycle bioassay with the amphipod shredder *Hyalella azteca* was performed with ten amphipods of random sex per replicate (n=30). A mixture comprising five current-use fungicides was tested at two environmentally relevant sum concentrations, namely 5 and 25 μg/L. Variables related to the energy processing (leaf consumption and feces production), growth (body length and dry weight), energy storage (lipid content), and reproduction (number of precopula pairs as well as number and length of offspring) of *H. azteca* were investigated. While leaf consumption was not affected by fungicide exposure, feces production was significantly reduced (~20%) in both fungicide treatments compared to the control, indicating an increased utilization of ingested food to counterbalance stress-related energy requirements. However, this mechanism did seemingly not entirely compensate for the presumed increased energy demand and might have induced trade-offs in the allocation of energy among different physiological processes: a reduced energy allocation towards maintenance is suggested by a significant 5-10% increase in mortality in both fungicide treatments compared to the control. Moreover, while growth as well as energy storage remained unaffected, abundance of precopula pairs was lower in both fungicide treatments (significantly only in the 25-μg/L treatment). Hence, time to first offspring release was delayed in both fungicide treatments and the number of offspring was significantly lower in the 25-μg/L treatment, whereas offspring length was unmodified. Our data indicate that long-term exposure to fungicides at environmentally relevant concentrations may impair shredders' reproductive performance, which may cause declining shredder abundances. Ultimately, this might reduce shredder populations' functional performance, namely their contribution to leaf litter breakdown, and may thus trigger negative implications in associated aquatic food webs.

Fish model species in human and environmental toxicology (I)

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Alterations of the bile acid synthesis, hepatic detoxifying mechanisms and lipid metabolisms in European sea bass (Dicentrarchus labrax) after in vivo exposure to a xenoestrogen mixture

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Xenoestrogenic chemicals are present in aquatic habitats and may feminize fish populations. Chemicals such as bisphenol A, alkylphenols and synthetic hormones are disposed in water masses by sewage effluent discharges negatively affecting the reproduction, growth and development of impacted fish populations. Most xenoestrogens are metabolized in the liver of fish and disrupt general hepatic metabolism and lipid homeostasis. In this work, we aimed to study the effect of a mixture of four well known xenoestrogens in the hepatic metabolism of juvenile fish. European sea bass *(Dicentrarchus labrax)* juvenileswere exposed to an equimolar mixture of bisphenol A, 4-*tert*-octylphenol, diethylstilbestrol and 17β-estradiol for 10 days, followed by a recovery period in clean water for additional 7 days. Chemical determination of xenoestrogens was performed in bile, blood and liver. Genes involved in bile acid synthesis, such as cholesterol 7α-hydroxylase (*cyp7a1*)*,* farnesoid-X-receptor (*fxr*)*,* liver-X-receptor (*lxr*)and *hmg-CoA reductase* were amplified and sequenced for qPCRs analysis. Likewise, genes involved in the detoxification metabolism pathway such as *cyp1a, cyp3a, udp* and *abcb11* were investigated as well as vitellogenin (*vtg*), a marker of xenoestrogenicity. A gradual change of bile color (from green to white) was observed over the exposure time. After the recovery period, the bile color reversed to green color. In addition, plasma clotting was common in exposed fish and chemical tissue distribution changed from high levels of xenoestrogens in the bile at the first days to high concentrations of BPA in liver and blood during the last days of exposure period and recovery time. Liver somatic index significantly decreased after 10 days of exposure. After 3 days of exposure c*yp7a1, fxr, lxr, cyp1a* and *udp* were down-regulated, whereas *hmg-Coar, cyp3a* and *abcb11* were not altered. After 7 days of exposure all these genes as well as *cyp3a* and *udp* were upregulated. No changes were registered in *vtg* levels. At the end of the recovery period, all assessed genes were significantly down-regulated compared to the transcription levels at day 7. Overall, it was concluded that the exposure to xenoestrogens induced a severe impact in liver metabolism impairing bile acid synthesis, detoxification mechanisms and the xenoestrogenic response. Funded by: Spanish MINECO and EU-FEDER (AGL2015-63936-R; CTM2014-56628-C3-1-R), Basque Government (IT810-13), UPV/EHU (UFI 11/37).

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Thyroid hormone disrupting effects in zebrafish exposed to compounds found in indoor environments

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The endocrine disrupting effects of estrogenic compounds like bisphenol A or the anticonception pill are well studied. But many more substances in our environment are expected to have endocrine disrupting potential. Despite the growing numbers of patients that are diagnosed with thyroid disorders, thyroid hormone disrupting effects of compounds are less investigated. Within this study, we assess the exposure situation to anthropogenic thyroid hormone disrupting compounds (THDCs) in homes. Key emerging THDCs are identified in serum samples from indoor pet cats and humans (including cord blood serum) as a model for the internal exposure to chemicals and in dust extracts as a route of external exposure. In the following the thyroid disrupting effects of these compounds individually and potential mixture effects are assessed. Therefore, individual compounds as well as artificial mixtures are studied by exposing zebrafish embryos. A variety of different endpoints during thyroid relevant stages of the early development are assessed. Endpoints were chosen based on literature. Eye size was measured, visual malformation, hatching, heartbeat, swimming behaviour and swim bladder inflation were monitored. 25 compounds were selected based on their detection in cat, fetal and maternal serum samples and dust extracts. Artifical mixtures were made based on the measured levels. All mixtures consist of different compounds. All tested substances caused a variety of different effects, with behavioral alterations being the most observed. A clear link between thyroid hormone distribution and the observed effects cannot be made yet. Therefore, more thyroid specific endpoints will be assessed in the future. Till this end, no synergistic mixture effects could be observed but more detailed analyses are following. Interestingly the toxicity of each mixture was driven by one individual compound. Our findings show a diversity of effects caused by compounds found in the home indoor environment and the usability of zebrafish to test substances for their potential human toxicity. Our results will be used to improve the understanding of human and child exposures to indoor related chemicals and their thyroidogenic effects in mixtures. The project is aimed to feed in scientific results for improved management of EDCs.

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The annual killifish Nothobranchius furzeri as a new model in long-term ecotoxicological research

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Given the obligation to perform long-term tests on vertebrates in ecotoxicological risk assessment and the high cost in terms of labour, money and time associated with such tests on current models, there is a need for new and efficient vertebrate model species to study chronic and multigenerational toxicity. *Nothobranchius furzeri*, the turquoise killifish, has two characteristics which make it into a good candidate model organism for long-term freshwater ecotoxicological tests. First of all, the species is characterised by a very short life cycle of ±12 weeks and second, it produces drought-resistant eggs which can remain viable in a state of dormancy up to several years. While the former characteristic facilitates multi-generational testing over a short time-span, the latter removes the need for a continuous culture of test organisms and enables synchronised exposure of the test population through controlled hatching. In order to test its validity as a new model organism, we tested the acute and chronic sensitivity of *N. furzeri* to several pollutants. In these exposure experiments, we wanted to determine the sensitivity of the species to a range of well-studied toxicants with different modes of action and compare its sensitivity range to that of current fish models. Furthermore, as exposure times may be much longer in natural compared to laboratory settings and effects may only emerge in later stages of an organism's life cycle, we studied long-term effects of sublethal concentrations of copper. In general, and on the basis of applied acute test concentrations, the sensitivity of *N. furzeri* appears to be comparable to or higher than the sensitivity of other model species. Chronic exposure of *N. furzeri* to copper showed increased lethal damage, decreased life span, slower maturation and reduced fecundity (Fig. 2) when chronically exposed to concentrations of ≥10.27 μg Cu/L. Furthermore, we observed a damage and repair mechanism, with fish either succumbing to the exposure during the first weeks, or developing defence and copper-excreting mechanisms to endure exposure on the long term, potentially trading off with fitness *Nothobranchius furzeri* is a suitable new model for ecotoxicological purposes. Since the species appears to be more sensitive compared

to traditional model species and has a short generation time it is especially suited for long term exposure and multi-generational assays, which are considered highly relevant.

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Development of a test method for transgenerational effects of genetically modified crops in food using the zebrafish model

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Ever since *genetically modified (GM) crops* were introduced, their safety regarding human consumption has been questioned. One of the main concerns is that the current test method, a 90-day rodent toxicity study, does not specifically assess effects on embryonic development or reproduction. Therefore, the aim of this study is to develop a *new method*, using the zebrafish model, to assess *transgenerational effects* of GM crops in food. Since *GM maize* was selected to develop the method, the first phase of this study was to define the *maximum percentage of maize* tolerable for zebrafish. Fish were fed for 4 weeks with 6 experimental feeds ranging from 0% to 25% *of non-GM maize*. Growth slightly (2.5%) decreased when fish were fed with 0% or 25% of maize. The hepatosomatic index (percentage liver weight/total body weight) of males significantly increased when fed with 20% or 25% of maize. Feed digestibility analysis showed a decrease in carbohydrate uptake when fish were fed with an increasing percentage of maize substitution. Based on these outcomes, we selected *15% maize* as the maximum tolerable percentage. Furthermore, it is important that any potential effect of a GM crop is interpreted relative to the *natural variation* that can be observed in response to feeding with non-GM varieties of the *same crop species*. We therefore fed zebrafish for 12 weeks with 10 different non-GM maize varieties (15%). We observed significant differences for the carbohydrate level in the liver of adult males, for the swimming behaviour of adults and larvae, and for the relative condition factor and length of larvae. These results highlight the importance of defining the natural response variation as even feeding with non-GM varieties can cause significant differences. Next, a *transgenerational* experiment was initiated to investigate whether the test system allows us to assess potential transgenerational effects. After 16 weeks of feeding with an experimental and a commercial GM maize and their corresponding non-GM controls (15%), no effects were observed. The variation between the GM condition and their controls was small compared to the natural response variation. Since there is no GMO available that causes known adverse effects due to the genetic modification itself, we opted to expose a subset of every generation to the well-known toxicant cadmium as a positive experiment control to monitor the sensitivity of the zebrafish over different generations.

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Applying Zebrafish as an integrative model in effects-directed analysis (EDA) to identify key drivers in Danube River surface water

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Zebrafish *Danio rerio*, owe to its initiatives in specific modes of action and mechanism-specific endpoints, it is perfect for EDA investigations to identify the contaminants responsible for the toxic effects in the environment. The aim of this study was to establish an integrative model with zebrafish which ranged from molecular, cellular (in vitro) to whole organism (in vivo) level to identify key drivers in the Danube River. The acute fish embryo test(FET), in vivo AhR-mediated activity assay, in vivo estrogenicity assay and in vitro genotoxicity assay were carried out to analyze the development toxicity, AhR mediated activity, estrogenic activity and genotoxicity of each RP- HPLC fraction. The result show that the effect presented relatively at different biologic level on their organism, rather than single independent level. The detected chemicals explained part of the biological effect for most endpoints and the specific effect of AhR, ER and genotoxic partly explained comprehensive developmental effects. The study was limited by the contribution of the detected biological effect to the the individual, population or ecosystem which is hampered by the limitation of long-term, whole-life and multi-generation fish assays.

Measuring and Estimating Dose Metrics: Linking Exposures to Effects for improved Chemical Risk Assessment

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Predicting chemical concentrations in the exposure medium: a validated model for plastic well plates

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Establishing a robust *in vitro* exposure system is still very challenging, particularly for hydrophobic and volatile compounds; thus, nominal concentrations are frequently still the only metrics reported for *in vitro* tests. For this reason, several modeling approaches for estimating chemical distribution in *in vitro* systems have been developed. Yet, there is lack of appropriate measurement data for the validation of these models. Additionally, most of the existing models do not account for plastic binding (very important for no or low serum concentration) and chemical evaporation in the *in vitro* system, the latter of which also depends on how the exposure system is sealed. Our aim was to develop and validate a simple computational model for predicting chemical concentrations in the exposure medium (with and without serum) at the end of the experiment. This model should also be valid for different medium volumes, well-plate sizes, testing objects, etc. The computational model has been developed based on the assumption that chemical decrease in the exposure medium depends on octanol-water partition coefficient ($LogK_{OW}$) and Henry's Law Constant (LogHLC). The model was then calibrated based on concentrations of 27 chemicals measured in 2mL exposure medium L15/ex in 24-well plate with RTgill-W1 cells and was validated based on 13 other chemicals measured in different studies for the same system. Model predictions were in a very good agreement with measured concentrations used for the model validation ($r^2 = 0.98$). The model has also been extrapolated to other systems, e.g. with different medium volume, serum content, well size and testing objects (RTgutGC, RTgill-W1, zebrafish embryos), for which it was also validated. As opposite to the use of nominal concentrations, there was no significant difference between EC50s determined in 1 and 2 mL exposure medium when predicted chemical concentrations were taken into account. In addition, the coefficient of determination between predicted and measured data for zebrafish embryos was also high ($r^2 = 0.88$). Our approach is the first step for accurate predictions of bioavailable chemical concentrations in the exposure medium in *in vitro* systems. Already in its current form, it can successfully be used for predicting chemical concentrations in L15/ex medium in 24-well plates after 24h exposures and for several other experimental set-ups.

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The influence of a surfactant's polar head group on its in vitro distribution and cytotoxic potency

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The *in vitro* distribution and the effect of this distribution on *in vitro* bioassay readout have been studied for neutral organic chemicals. The octanol water partition coefficient (K_{OW}) and Henry's law constant correlate with a chemical's bioavailable fraction *in vitro*. The partitioning behavior of charged, complex chemicals like surfactants in *in vitro* assays has not been well studied. The aim of this study was to measure the fraction of cationic and anionic surfactants with alkyl chains of eight and twelve carbons in exposure medium and well plate plastic over time in an RTgill-W1 basal cytotoxicity assay. The extent to which a surfactant's headgroup, charge, charge shielding, pKa and membrane-water partition coefficient (K_{MW}), as well the extent to which pH, serum and calcium levels of the exposure medium influence the partitioning behavior and cytotoxicity readout was assessed. Results indicate that cytotoxic effect concentrations were dependent on the chosen dose metric (measured medium concentrations after exposure versus nominal) and exposure time. There was a large variation in cytotoxic potency between surfactants with varying head groups, where cationic surfactants, especially surfactants with a permanently charged head group, were found to be most cytotoxic likely due, in part, to the mechanism of membrane insertion. Given this variation in partitioning behaviour, the nominal effect concentrations used to rank surfactants according to their cytotoxic potency across assays will not reflect the ranking of toxic potencies based on bioavailable or cell-associated effect concentrations.

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The chemical exposure toxicity space (CETS) model: expanding its application to non-fish organisms including Hyalella Azteca and Lumbriculus variegatus.

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The Chemical Exposure Toxicity Space (CETS) model was introduced at SETAC Orlando in November, 2016 as a tool to illustrate plausible test conditions for aquatic toxicity tests for fish. It was successfully applied to experimental toxicity test data for guppies and several organic narcotic chemicals, showing it can be a valuable tool for preventing excessive or unproductive toxicity testing. As an extension to that study we have applied the model to two other organisms, *Hyalella Azteca* and *Lumbriculus variegatus*, using experimental toxicity test data [1]. A full description of the model is currently in press and has been accepted for publication by the Journal of Environmental Toxicology and Chemistry. The CETS plot is designed to aid in selection of chemical concentrations in water, test durations, and for interpreting test results. This has been prompted by three recent studies, a modeling study explaining the difficulties in conducting bioassays for super-hydrophobic substances such as siloxanes [2], an experimental study in which high-quality critical body residues were obtained for multiple species and chemicals [1], and a study by Thomas and colleagues discussing the merits of the chemical activity approach for assessing aquatic ecotoxicology data for organic chemicals [3]. In this extension to our study we applied the same version of the CETS model to the Lumbriculus and Hyalella organisms' toxicity data. Graphs of water concentration or activity vs a non-dimensional time term (Φ) resulted in a curve showing lethal concentrations (or activities) as a function of time, Φ. Graphs of these results show the experimental data generally falling on and on either side of the predicted data for both species.The results indicate that with the exception of some outliers the CETS model is a very good indicator of lethality for the non-fish organisms tested. This suggests that the current CETS model may be used to predict toxicity test conditions for non-fish species, providing guidance on toxicity test parameters as well as a general understanding of the effects and limitations that hydrophobicity and solubility have on toxicity tests. It is hoped that the CETS model will be used to reduce animal usage in toxicity testing. Future developments of the CETS diagram may include an aquatic dietary exposure model. [1] *Environ Sci Technol* 2015, 49:1879-1887 [2] *Environ Sci Technol* 2015, 49 (19) 11913-11922 [3] *Environ Sci Technol* 2015, 49:12289-12296

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Advancing TKTD modelling of developmental toxicity: Quantifying glutathione dynamics in the organogenesis stage rat embryo

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A quantitative understanding of glutathione:glutathione disulfide (GSH:GSSG) redox dynamics is essential to develop ToxicoKinetic-ToxicoDynamic models for developmental toxicity. The GSH:GSSG balance affects a variety of basic cellular functions (e.g. proliferation, differentiation and apoptosis) that are critical to ensure a normal embryonic development. Many teratogens elicit their toxic action by perturbing this redox balance causing malformations, behavioral deficits and/or lethality. Precise control of the GSH:GSSG redox balance is vital for the developing embryo, but a quantitative, systems-level understanding of conceptal redox dynamics is presently lacking. We develop a dynamic mass-balance model for overall GSH metabolism in the organogenesis stage rat conceptus that accounts for the mass-fluxes of GSH precursor uptake, utilization, turnover, and enzyme kinetics. Two initial challenges were confronted: First, rapidly growing embryos (EMB) can create a considerable 'growth dilution' if not accounted for, and second, GSH:GSSG dynamics are spatially-segregated where the visceral yolk sac (VYS) and EMB proper retain their distinctly separate redox dynamics. To address these challenges, we obtained consistent time-course data of relevant growth parameters in rat whole embryo cultures for VYS surface area, size, volume, and the concentrations of GSH, GSSG, cysteine (Cys), and cystine (CySS) in the VYS, yolk sac fluid (YSF), amniotic fluid (AF), and in the EMB proper. We observed significant exponential growth for the VYS surface area (r^2=0.97), VYS protein synthesis (r^2=0.82) and EMB protein synthesis (r^2=0.89). Our results show that concentrations of GSH and GSSG remain remarkably stable during the organogenesis period in VYS and EMB, indicating that GSH:GSSG is maintained at steady-state levels in both tissue compartments, albeit at different levels. A mass-balance model consisting of 21 coupled differential equations describing transport fluxes, enzyme kinetics, biosynthesis and growth dilution of 8 metabolites (incl. GSH, GSSH, Cys, CySS) in 3 compartments (VYS, YSF+AF, EMB) was developed. Initial results show good overall agreement between model predictions and measurements. Our model provides insights into the mass-fluxes necessary to maintain steady-state levels of GSH:GSSG in the VYS and EMB of the rat embryo during normal development and is essential to develop mechanistic TKTD models for developmental toxicity caused by oxidative stress.

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Future water monitoring - steps towards a panel of effect based tools based on adverse outcome pathways

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Chemicals in the aquatic environment occur in mixtures rather than as individual entities. Environmental quality monitoring is thus posed with the challenge to comprehensively assess a multitude of potential compounds and potential adverse effects. Component-based mixture toxicity consideration and the potential relevance of low individual compound concentrations therein have been demonstrated in pioneering studies by Könemann (1980) and Hermens and Leeuwangh (1982). Effect-based tools have been suggested as complements to chemical analytical characterisation of complex pollution patterns. Here we present a 'round robin study' with 17 different bioanalytical tools analysing the same sample in dilution series. The 'synthetic' sample comprises of 12 organic water pollutants of diverse structure and modes of toxic action in two different concentration ratios. All assays ran dilution series testing of the components that can be anticipated as major effect drivers, which allowed mixture modelling on the complete sample even without knowing all individual components activities. For several assays it could be demonstrated that they detect their suspected active components on the background of a complex sample consisting of many contaminants. Thus these bioassays can be expected to be fit for specific environmental monitoring. Also, the majority of bioassays were able to detect the combined effect of the specifically bioactive compounds against a complex contaminated sample background which gives confidence to progress with the development of effect-based water quality monitoring. Moreover, we could demonstrate that the different bioassays showed a differentiated picture on the jointly investigated sample. This means that based on our study we can proceed to develop bioanalytical tools for two distinct monitoring applications (i) compiling comprehensive effect-based bioassay panels for water quality assessment and (ii) designing tailored surveillance tools to safeguard specific water abstraction purposes. **References** Könemann H. 1981. Fish toxicity tests with mixtures of more than two chemicals: a proposal for a quantitative approach and experimental results. Toxicology 19: 229-238. Hermens J, Leeuwangh P. 1982. Joint toxicity of 8 and 24 chemicals to the guppy (Poecilia reticulata). Ecotox Environ Saf 6: 302-310.

Organic micropollutants in the environment: analytical challenges and engineering innovations (III)

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Determination of Phthalate Esters and Personal Care Products in Fish Tissues with Ultra-performance Liquid Chromatography/tandem Mass Spectrometry

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Phthalate esters (PAEs) and personal care products (PCPs), such as analgesics, UV filters, cosmetic preservatives, caffeine, and DEET are used at a large scale, and they have been found ubiquitous in the aquatic environment. This study developed an analytical method for six PAEs and 11 PCPs in fish tissues (muscle, liver, gonads, and eggs). Samples were treated with matrix solid-phase dispersion (MSPD) on SiliaBond C18, and analytes were eluted with methanol and acetone; the eluents were passed through acidic alumina cartridges for cleanup, then were concentrated to barely dry. Reconstituents of 100 μL methanol were analyzed with ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) using electrospray ionization (ESI) at multiple reaction monitoring (MRM) mode, and analytes were quantified with isotope-dilution techniques. PAEs and five basic PCPs were separated on an Ascentis Express F5 column (30 × 2.1 mm, 2 μm) with mobile phases of 5 mM ammonium acetate$_{(aq)}$ and methanol, which provided 1.9-48 times higher signal intensities comparing with acetonitrile, and offered baseline separation of di(2-ethylhexyl) phthalate (DEHP) and di-*n*-octyl phthalate (DNOP), both yielding the ion transition of m/z 319.3 > 148.9. Trace amount of DEHP (191 pg/mL), diisononyl phthalate (DINP; 229 pg/mL), and diisodecyl phthalate (DIDP; 17 pg/mL) were observed in the LC-MS grade methanol; therefore, an ACQUITY UPLC Isolator column (50 x 2.1 mm, 3.5 μm) was installed onto the UPLC system to eliminate the backgrounds of PAEs from mobile phases and the instrument. Six acidic PCPs were separated on a CORTECS C18 column (30 × 2.1 mm, 1.6 μm) with 0.04% acetic acid$_{(aq)}$ and methanol for gradient elution. Ues of methanol and acetone eluted analytes better from SiliaBond C18 (most 30-64%) than those using methanol and dichloromethane (most 12-66%), and the effectiveness of 5 mL at each portion (four portions in the total) were similar to those with 7.5 mL. These tests were applied on alumina cartridges as well, and recoveries of analytes from alumina cartridges using methanol and acetone (most 40-68%) were also similar or better than those using methanol and dichloromethane (most 30-66%). The recoveries of the analytes through the whole process were most 25-70%. Analyte concentrations in tissues from more than 40 fish will be presented.

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Determination of opioid analgesics and their metabolites in municipal wastewaters by liquid chromatography-tandem mass spectrometry

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The major sources of opioid compounds to the aquatic environment are human excretion and dumping of unused medications to the sewer. The existing analytical methods include rather limited number of opioid analgesics (OA) representatives and/or their major human metabolites while the methods that comprise a broad spectrum of OAs are still missing. Therefore, the aim of this work was to develop a multiresidue LC-MS/MS method for the simultaneous determination of trace levels of 28 OAs in municipal wastewaters. The method development included: A) optimization of chromatographic and mass spectrometric conditions for the separation and detection of target analytes, respectively; B) optimization of extraction procedures for the dissolved and particulate OAs; C) full validation of analytical protocols for untreated wastewater and secondary effluents and D) the method evaluation for real wastewater samples. In order to optimize chromatographic separation different columns (Synergy Polar, Gemini C18 and ACE C18-PFP) and eluents were evaluated. At the final chromatographic conditions, a complete chromatographic separation of all analytes was achieved by using Synergy Polar column with methanol and water, both containing 0,1% HAc, as eluting solvents. The enrichment of opioid analgesics from aqueous samples and suspended solids were performed employing solid-phase extraction (SPE) based on mixed cation-exchange (Oasis MCX) cartridges and pressurised liquid extraction (PLE), respectively. During extraction optimisation different SPE cartridges were investigated (Oasis MCX and Oasis HLB) in order to optimise the overall recovery and to reduce the matrix effects. The optimal extraction efficiency of dissolved OAs was obtained by using Oasis MCX cartridges (recoveries: 43-81%). For the extraction of particulate phase ultrasonic extraction and PLE as well as different solvents were tested. The best results were achieved by using PLE with 1% ammonia solution in methanol as a solvent. Recoveries were in range from 72-144% for almost all opioids with exception of 6-acetylmorphyne and glucuronides (15-46%). The method was applied for the assessment of OAs in municipal wastewater from Croatia. Most of the OAs were found in relatively low concentrations ranging from 1-50 ng/L. However, some analytes, such as tramadol, morphine, codeine and methadone showed significantly enhanced levels. The total concentration of tramadol and its metabolites exceeded 1 μg/L. Keywords: opioid analgesics, LC-MS/MS, wastewaters

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Tracing micropollutants in raw and drinking water in Sweden
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A multi-residue method was developed for detection of a wide range of micropollutants at sub ng L^{-1} levels in raw and drinking water – from source to tap. The method includes large volume sampling and extraction combined with liquid chromatography - high resolution mass spectrometry (LC-HRMS). It was used to analyse ~130 micropollutants (pesticides, pharmaceuticals, drugs, per- and polyfluoroalkyl substances (PFASs), and other pollutants) in water samples collected along a raw water path, inside a drinking water treatment plant (DWTP), and in the water distribution system. Out of the 134 target compounds, 38 were detected in at least one sample. The concentrations of individual substances ranged from sub ng L^{-1} levels to almost a 100 ng L^{-1}. The removal efficiency in the drinking water treatment plant was poor for most of the detected substances with an average removal of ~10%. The highest concentration for the treated drinking water was found for diclofenac followed by other pharmaceuticals (carbamazepine, cotinine, lamotrigine and tramadol), PFOS and caffeine. Similarly, in a national database survey of micropollutants in Swedish raw and drinking water, the top-11 substances showing both high detection frequency and wide geographical distribution included mostly pharmaceuticals (fluconazole, irbesartan, carbamazepine, naproxen, paracetamol, and venlafaxine) and in addition PFASs (PFBS, PFHxS, PFOA, PFOS) and BAM (2,6-dichlorobenzamide). In the field study and the database survey, only well-known micropollutants were targeted. The way forward for widening the scope of detecting potentially hazardous micropollutants in raw and drinking water is to conduct suspect screening using LC-HRMS. We previously developed a suspect screening method that involves a PMT scoring approach, thus considering persistence (P), mobility (M), and toxicity (T) of the potential candidate substances. Additionally, usage data are considered through data from the Swedish Chemical's Agency. It has proved to be an easy to use, flexible tool for navigating towards top-relevant micropollutants among tens of thousands candidate substances. It is used in parallel with target screening to search for less known substances and can reveal emerging threats in raw and drinking water. The method is currently being tested in a field study investigating the raw and drinking water of seven DWTPs.

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Screening for pharmaceuticals in wastewater and receiving seawater in Nuuk, Greenland
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Pharmaceuticals are micropollutants that over the last 15 years have received increasing attention. However, little information is available on the pollution levels of pharmaceuticals in arctic and sub-arctic environments. In Nuuk, the capital and largest city of Greenland, all industrial and domestic wastewater is discharged untreated in the surrounding sea. In this study, a broad screening for 205 pharmaceuticals in four wastewaters (including a hospital wastewater), a landfill percolate and their receiving seawaters in Nuuk, Greenland, is performed using a recently developed suspect screening method based on ultra-high performance liquid chromatography (UHPLC) and Orbitrap high-resolution mass spectrometry (HRMS). This technique enables full-spectrum analysis of a virtually unlimited number of analytes at trace levels, which raises the possibility for post-acquisition screening when no reference standards are a priori available. The screening takes into account both the accurate mass of the mono isotopic ion and up to three isotopes and their isotope ratios, as well as a peak/noise filter in a multivariate statistical model. This powerful screening, which is able to automatically detect peaks and match isotopes, guaranties a false negative rate of only 5%. This study demonstrates the applicability of our screening and provides one of the first occurrence data for pharmaceuticals in Nuuk. For screening quality control, all samples were spiked with a standard solution containing 71 pharmaceuticals and pesticides. False negative rates close to the expected level of 5% were obtained, which shows that the screening model, which has been calibrated on another dataset of wastewater samples, remains valid. The obtained false positive rates for the different types of water matrices were in the range of 0.7-1.4% and < 0.1-1.4%, when using the mono isotopic ion in combination with 2 and 3 isotopes, respectively. A total of 30 out of 205 pharmaceuticals were retained in the 10 samples from which 8 out of 12 in-house pharmaceuticals could be confirmed. Considering the obtained median limits of identification of about 2 $\mu g\ L^{-1}$ for wastewater and the applied sample loading volumes, the concentrations of these detections are likely in the high (>100) ng L^{-1} for seawater and $\mu g\ L^{-1}$ level for wastewater.

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Poster spotlight: TH094, TH095, TH097, TH116

Hazard and exposure assessment of chemical mixtures: steps towards increasing the realism of human and ecological risk assessments

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Transthyretin-binding activity of complex mixtures representing the composition of house-dust contaminants in dust and serum
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Ingestion of house dust is a major route of human exposure to thyroid hormone disrupting compounds (THDCs). One of the mechanisms by which compounds may act as THDCs is through competition with thyroid hormone thyroxin (T4) for binding to its distributor protein transthyretin (TTR). In the current study, we determined the TTR-binding capacity of 25 house-dust contaminants or their metabolites in four different complex mixtures, reflecting the composition (based on reported median concentrations) in dust, in human cord blood serum, in human adult serum, and in cat serum. The aim of the study was (1) to determine if the TTR-binding potency of the complex mixtures was properly described by concentration addition, (2) to estimate what the actual TTR-binding capacity is of the mixture of TTR-binding compounds to which humans and cats are internally exposed, and (3) to determine which compounds are driving the TTR-binding capacitiy in these complex mixtures. A test set was composed consisting of 25 compounds belonging to different chemical classes, i.e. perfluorinated compounds, polyhalogenated organic hydrocarbons and their metabolites, and other halogenated and unhalogenated phenols. Selection of compounds was based on three different criteria, i.e. presence in house dust, predicted or experimentally confirmed TTR-binding capacity, and reported concentrations in dust, cord blood serum, adult serum, or cat serum. TTR-binding capacities of the four different mixtures and the 25 individual compounds were determined in an in vitro binding assay with human TTR and a fluorescent T4-probe. Concentration-dependent TTR-binding activity was observed for all 25 individual compounds and for the four mixtures, with no deviation from concentration addition. For the median concentrations of house dust contaminants actually determined in serum, the margin of exposure to the in vitro IC_{50} for TTR-binding ranged from a factor 40 for the cat serum mixture to only a factor 2 for the human serum mixtures. Since the selected set of test compounds does not completely cover the complete spectrum of TTR-binding (metabolites of) house dust contaminants, the actual margin of exposure is probably even smaller. Out of the 25 housedust contaminants, pentachlorophenol and PFOS were the main drivers for the TTR-binding activity of the human serum mixtures. In cat serum, 2,4,6-tribromophenol and 6OH-BDE-47 were also major contributors.

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Different responses of zebrafish and human-based estrogenicity bioassays to selected environmental contaminants and their mixtures
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Effect-based tools (EBT) based on estrogen receptor (ER) activation are widely used to monitor xeno-estrogens in environmental samples. We previously reported that established human (MELN cells, expressing hERα) and zebrafish (ZELHα cells expressing zfERα, ZELHβ2 cells expressing zfERβ2) reporter cell lines are robust, reliable and sensitive to detect well-known ER ligands. However, they can respond to surface water and effluents samples differently, with samples being selectively active either on human or zebrafish cells. Different responses may be due to non-identified active substances and/or interactions with other chemicals, as ER activation depends on the intrinsic potency of the chemical and on the cell context (e.g. metabolism). Therefore, the present study aimed to compare the responses of zebrafish and human EBT to selected environmental pollutants, alone and in combination. Interactions between chemicals were taken into account by (1) assessing both activation and inhibition of ER response, (2) using concentration addition (CA) model to predict additive effects, and (3) combining active and non-active pollutants. While genistein and bisphenol A (BPA) were active on all EBTs, marked differences were observed for other chemicals. Triphenylphosphate (TPP) and benzo(a)pyrene (BaP) induced ER activation in MELN cells but decreased E2 response in zebrafish cells. Chlorophene, benzo(b)fluoranthene and propiconazole selectively inhibited the E2-induced reporter gene response in zebrafish cells. The first 12-component mixture results indicated that the effects in ZELHα and ZELHβ2 cells were driven by inhibiting chemicals (TPP and propiconazole), while the estrogenic activity of BPA and genistein was not detected, although predicted. In MELN cells, the estrogenic response (driven by genistein, BPA and TPP) agreed overall well with CA prediction. Work is ongoing with other mixture designs. Altogether, the results indicate that both the cell context (fish vs human) and the effects of anti-estrogenic chemicals influence ER response. In line with previous observations on environmental samples, our study raises the question of cross-species differences and fish-specific effects, highlighting the importance of the cell model for water quality biomonitoring. Future work will focus on the refinement of estrogenicity assessment by taking into account anti-estrogenic effects in environmental samples. This work was performed within the EU FP7 project SOLUTIONS.

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Toxic mixtures in time - the sequence makes the poison

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It is generally agreed that "the dose makes the poison" – that chemicals can be toxic or non-toxic depending on their dose. This principle assumes that once a chemical is cleared out of the organism (toxicokinetic recovery), it no longer has any effect. However, it overlooks the other more subtle process of re-establishing homeostasis, toxicodynamic recovery, which can be fast or slow. We tested four combinations of substances and found a clear difference in toxicity when the exposure order of two toxicants was reversed, while maintaining the same dose. When toxicodynamic recovery of the organism was slow relative to the interval between exposures it resulted in carry-over toxicity and so caused this sequence effect. We provide evidence of carry-over toxicity amongst chemicals acting on different targets and when exposure is several days apart. It is therefore not only the dose that makes the poison but also the exposure sequence.

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A non-toxic aquatic environment: a Paracelsian exploration of European aquatic mixture impacts

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The aim of environmental management has been a non-toxic environment, as recently reconfirmed in the 7th European Environmental Action Plan. In the Paracelsian view, everything is toxic, but it is the concentration that would make the environment non-toxic. Moreover, to reach a Good Ecological Status, neither mixtures nor other stress factors should affect ecosystems. So: what's the safe concentration of ambient mixtures, given the fact that mixtures of pollutants occur everywhere? And can ecotoxicological and ecological approaches be bridged to handle multi-stress? This contribution presents the combined use of predictive impact models and of diagnostic field observations, so as to provide mixture- and multi stress approaches for decision support. The results originate from the European project SOLUTIONS and a Dutch project on Ecological Key Factors in surface water assessment and management. First, based on monitoring data and diagnostic analyses, current pollutant exposures imply the presence of mixture impacts, varying in space and time. There is evidence for an exposure-dependent main effect of mixtures, with latitude for deriving a non-toxic status boundary. The contributions of individual compounds to impacts appeared highly skewed. Second, modeling exercises were undertaken, starting from production masses of more than 13,000 chemicals. Produced and emitted masses were evaluated, so as to yield predicted concentrations of chemical mixtures in an 'average European water body'. Thereupon, the toxic pressures of the individual compounds and the next mixture were derived for that water body. Again, it appeared that the relative contributions of chemicals to the mixture was highly skewed. The repeated finding that mixture effects are likely attributable to a small fraction of pollutants – be it of different composition across sites – provides an option to focus research and management efforts. The contribution touches upon three novel insights. One bridges the gap between ecology and ecotoxicology in the context of analyzing the integrity of aquatic ecosystems. A second one sketches how the large ecotoxicity data gap might be solved, as the ambition was to enable impact prediction ofl all 13,000 chemicals. A third one shows that the receiving ecosystems are never "the" virtual ecosystem that often appears in the definition of the so-called policy protection endpoint, but that the vulnerability of each system varies across sites.

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Poster spotlight: TH128, TH129, TH130, TH132

Human health: use of biomonitoring data as validation of high tier human exposure models

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High-selenium lentils in the daily diet to counteract health effects of chronic high arsenic exposure in Bangladeshi people

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According to the WHO, up to 100 million people worldwide are chronically exposed to dangerously high concentrations of arsenic (As) in their drinking water and food supply. Bangladesh has large areas where the groundwater has high As. Since this contamination of the tube well water was discovered in the 1990s, approximately 45 million Bangladeshis remain at risk from As concentrations greater than the WHO guideline value of 10 μg/L (ppb) (WHO Fact sheet N°372, December 2012) and greater than the Bangladeshi limit of 50 ppb. Selenium (Se), an important nutritional trace element can antagonize As, so efforts to relieve arsenicosis (chronic arsenic poisoning) have included daily treatment with Se pills. This would pose additional costs to poor families, and many people, children especially, do not tolerate or comply with daily pill consumption. In a 6 month clinical trial, we investigated whether high-selenium lentils could be a whole food solution to improve the health of arsenic-exposed villagers in Bangladesh. The study engaged 443 participants in two treatment groups. All participating households had arsenic levels in their tube well water ≥ 100 ppb, but over 50% were >250 ppb. In this double-blind study, one group daily consumed high-selenium lentils from the Canadian prairies, the other, low-selenium lentils grown in another ecozone. At the onset, mid-term, and end of the trial, samples (blood, urine, stool, hair) are collected, and health examinations include testing lung inflammation, body weight and blood pressure. As well, fortnightly morbidity questionnaires were conducted with the heads of household by the hired village healthcare workers. Results on morbidity, physical health indicators, arsenic excretion in urine and feces, and arsenic deposition in hair will be presented.

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Assessing multimedia/multipathway exposures to inorganic arsenic at individual level using MERLIN-Expo

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We report on model simulations performed using the newly developed exposure tool, MERLIN-Expo, in order to assess inorganic arsenic (iAs) exposure to adults resulting from past emissions by non-ferrous smelters in Belgium (Northern Campine area). Exposure scenarios were constructed to estimate external iAs exposure as well as the toxicologically relevant arsenic species (tAs, i.e., iAs, MMA and DMA) body burden in adults living in the vicinity of the former industrial sites as compared to adults living in adjacent areas and a reference area. Model simulations at individual level are compared with biomonitoring data from a Belgian monitoring campaign (Flemish Government (2008); Van Holderbeke et al., 2008) in order to verify the model performance of MERLIN-Expo when simulating complex scenarios that account for subject mobility, i.e., residence time at different locations (both indoors and outdoors) with varying exposure levels in the vicinity of the hot spots, and individual food consumption patterns. Adopting an assessment approach at individual-level improves on more commonly performed generic exposure assessments at population level, by including intake of iAs from local and purchased food products, and taking into account the mobility of participants, mobility between areas, which results in a more comprehensive assessment of individual recent intake of inorganic arsenic. The model predictions for individual adults under-predict the biomonitoring data by 7% on average, but with more important under-predictions for subjects at the upper end of exposure. Modelling exposure at individual level allows risk assessors to attribute the relative contribution of different exposure routes at various locations, reconstructing the exposure history for each individual. These results constitute a first and partial verification of the model performance of MERLIN-Expo when dealing with iAs in a complex site-specific exposure scenario, and demonstrate the robustness of the modelling tool for these situations.

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The DustEx model for calculating human exposure to semivolatile organic compounds (SVOCs) in dust: Evaluation and validation
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On average adults spend 86% and infants and toddlers 88% of their time indoors, where they are continuously exposed to dust. Since indoor monitoring shows that semi-volatile organic compounds (SVOCs) released indoors may transfer into house dust, contact of residents with house dust leads to exposure to these substances. In particular, for low-volatile substances released by using consumer products and articles, house dust is often speculated to be an important pathway of exposure. This project therefore aimed to systematically analyse the importance of house dust as a pathway of human exposure to chemicals from consumer products. In the DustEx project the relative contribution of the dust ingestion pathway was systematically evaluated by using a model framework consisting of a combination of published models, which describe the transfer of substances from consumer products into the indoor environment (DustEx model). The exposure of consumers via the dust pathway was calculated and compared to other exposure pathways such as inhalation and dermal absorption from air. Biomonitoring was used as a plausibility check for the internal exposure estimates. The development and evaluation of the DustEx model was supported by a small-scale field study under controlled conditions. In this study both environmental monitoring and biomonitoring were conducted. Deuterium-labelled semi-volatile organic compounds (SVOCs), four adipates and four phthalates were embedded in artificial consumer products and placed into regular inhabited apartments. Indoor air and settled dust was sampled regularly to assess transfer into these media under realistic conditions. Two measurement campaigns were conducted: The first campaign had enrolled five apartments for twelve weeks. In the second campaign three apartments selected from the previous five were investigated for eight weeks with a more detailed sampling scheme for dust, and at regular intervals spot urine samples were taken from at least one inhabitant per apartment and analysed for two of the nine substances. For the evaluation and validation of the DustEx model the respective SVOCs were modelled and the estimates for environmental concentrations compared to the environmental monitoring. In addition, the biomonitoring results for two substances were compared to systemic human exposure estimates based on dust ingestion. The evaluation showed that the DustEx model is suitable for estimating consumer exposure to substances in dust.

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Internal dosimetry metrics for risk assessment of endocrine disruptors - the case of bisphenol A
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A major outcome of INTEGRA project (CEFIC LRI) is a computational platform that integrates multimedia environmental, (external) exposure and toxicokinetic modelling. Use of toxicokinetic modelling advances both exposure and risk assessment of environmental chemicals since it allows internal dosimetry metrics based risk characterization. In this way high throughput system data such as the ones generated by Tox21 *in vitro* testing can be used, providing the essential tools for "exposure based risk assessment". This opens the way towards a higher level of assessment that incorporates refined exposure (tissue dosimetry) and toxicity testing (Biological Pathway Altering Dose – BPAD). The applicability of INTEGRA was tested on bisphenol-A. Several exposure scenarios were investigated, as well as exposure estimates were based from exposure reconstruction of real life EU-wide human biomonitoring data using exposure reconstruction algorithms. Reconstructed exposure was then run in forward mode in the PBBK model, so as to estimate the Biologically Effective Dose (BED) in the target tissue. In order to associate the risk of the several exposure scenarios based on BED derived by the PBBK model, two different exposure metrics were used: - The temporary Tolerable Daily Intake (t-TDI) of 4 μg/kg_bw/d proposed by the European Food Safety Authority, was translated into internal exposure, found to correspond to a concentration of 0.013 μg/L of free plasma BPA. - *In vitro*, the ToxCast assays provided six ER agonist or binding AC50 values for BPA, ranging from 0.6 to 1.7 μM. To calculate a conservative Biological Pathway Altering Dose (BPAD), the lowest ToxCast AC50 was selected (0.64 μM for Attagene Factorial cis ERE assay). Using internal dosimetry metrics, allowed us to identify bioavailability differences associated to (a) age (ontogeny of the enzymes related to glucuronidation), (b) route of exposure (effect of first pass metabolism) and (c) inter-individual susceptibility, associated to various polymorphisms of glycuronidases. The assessment indicated that taking into account age dependent bioavailability differences of BPA, specific exposure scenarios (i.e. bottle fed neonates and premature infants hosted in intensive care units) result in BED close to the EFSA t-TDI legislative threshold. Use of the ToxCast AC50 BPAD as an internal exposure risk characterization metric, resulted in increased margins of safety compared to conventional exposure/risk characterization.

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Poster spotlight: TH153, TH154, TH155, TH157

Microplastics, nanoplastics and co-contaminants: Fate, effects and risk assessment for biota, the environment and human health (II)

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Mechanical fragmentation of ingested microplastics by Antarctic krill
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Plastics are present in marine environments worldwide. The increased production and use of plastics, coupled with their longevity, suggest that the abundance of plastic debris including microplastics in the ocean is likely to increase. Planktonic filter feeders may be most at risk of microplastic ingestion, particularly to polyethylene, polypropylene and some polystyrene, which all have a specific density less than seawater making them buoyant and available to planktonic species. In this study we examined the physical effect of ingestion of PE microbeads, a common model microplastic, using Antarctic krill. These planktonic crustaceans are endemic to the Southern Ocean, and display considerable flexibility in their diet. Further analysis into tissue localisation of the ingested particles was also carried out. Microplastics were observed in faecal pellets, within the oesophagus, stomach, digestive gland and mid gut of deceased krill. Faecal pellets and whole digested krill contained a mixture of whole beads and bead fragments, indicating that the Antarctic krill were physically altering the beads after ingestion. Fragments vastly outnumbered whole beads in all images of filtered and digested krill. Depuration of microplastics by krill was size dependant, with small fragments slower to depurate than whole beads. Complete fragmentation of all ingested beads did not occur. Whole beads were found in the stomach and midgut content, as well as in faecal pellets; with exposure concentration appearing to play a role in the ability of krill to fragment the plastic. This phenomenon has not been observed in other planktonic crustaceans such as copepods or isopods, many of which have similarly developed gastric mills and mouthparts designed for chewing and breaking. There is an urgent need for data on microplastic ingestion in wild caught zooplankton, to determine if this phenomenon is occurring in the natural environment.

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Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclopina nana
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Microplastic pollution causes a major concern in the marine environment due to their worldwide distribution, persistence, and adverse effects of these pollutants in the marine ecosystem. Despite its global presence, there is still a lack of information on the effect of microplastics on marine organisms at the molecular level. Herein we demonstrated ingestion and egestion of nano- (0.05 μm) and micro-sized (0.5 and 6 μm) polystyrene microbeads in the marine copepod *Paracyclopina nana*, and examined molecular responses to exposure to microbeads with *in vivo* endpoints such as growth rate and fecundity. Also, we proposed an adverse outcome pathway for microplastic exposure that covers molecular and individual levels. This study provides the first insight into the mode of action in terms of microplastic-induced oxidative stress and related signaling pathways in *P. nana*.

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Rapid filtering and ingestion of microplastics by marine mussels under ecologically-relevant scenarios
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Plastic debris in marine ecosystems is one of emergent problems that our plastic-addicted society faces. Plastic over-consumption and the lack of management policies is reflected in the ubiquity of plastic microparticles in oceans, with potential consequences to the biota. In order to evaluate the effects of plastic

microparticles on marine filter feeders, the present work, deploying the Mediterranean mussel as model, comprises three interlinked aims: i) estimating plastic microparticle clearance rate through filter-feeding; ii) tracing the presence of microparticles in organisms, and iii) determining effects of particle ingestion. Mussels were exposed to two polystyrene microparticles with different sizes (6 um and 10 um), single and combined, at an environmentally-relevant concentration (1000 part.ml^{-1}), in a series of short and mid-term bioassays. The biological effects of exposure were chiefly determined histopathologically. The findings indicate that the animals are able to rapidly remove both particles from the water column. Interestingly, the larger particles could be found in digestive tract just after 5 min of exposure, whereas the smaller particles required about 15 min. In either case, and in the mixed exposure, particles were only found in the lumen of gut (and faeces) and not in gills or the digestive gland. No traces of microplastics were found in the remaining visceral mass. Regardless of particle, singly or combined, no severe internal lesions were found, including abrasion of digestive epithelia, even after 21 days. However, focal haematocytic infiltration was observed in the same epithelia, without clear time-responsiveness. The results indicate that the animals are indeed able to rapidly remove the particles from the water column and transfer them into the digestive tract. Here, they accumulate and eventually generate moderate inflammatory-like responses, at least during the time of the bioassays and under ecologically-relevant concentrations of microplastics. In spite of the reduced internal damage, the fact that the animals are able to swiftly translocate plastic particles to the gut reveals that filter feeding organisms are indeed a target of concern. Altogether, the study raises further concerns on microplastic risk, with particular respect to realistically long exposures to micromaterials that even such specialised filter-feeders as bivalve molluscs are unable to discriminate.

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Tissue alteration in blue mussels (Mytilus galloprovincialis) caused by the ingestion of polyethylene microplastic particles from toothpaste
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Polyethylene (PE) microplastic particles (< 5 mm) found in certain consumer products, such as toothpaste, find their way into the marine environment via effluents from wastewater treatment works. There is serious concern that these particles may harm marine organisms, particularly filter feeders such as bivalves. In order to evaluate the effects of such particles on bivalves, blue mussels (*Mytilus gallloprovincialis*) were exposed over 21 days to PE particles (10 mg/L; 50-570 μm) isolated from toothpaste. Both virgin and weathered PE particles (deployed in the Outer Oslofjord for 3 weeks) were used to best mimic environmentally realistic exposure scenarios in marine waters. The mussels ingested both virgin and weathered particles, but 26% more of the weathered particles. Particles < 383 μM in size were ingested. To our knowledge, this is the first study that identifies the ingestion of primary microplastic from consumer products in bivalves. PE particle ingestion resulted in structural changes to the gills and digestive gland and necrosis in other tissues such as the mantel.

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Gene expression in liver of European sea bass Dicentrarchus labrax experimentally exposed to PVC microplastics
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Plastic debris, in particular microplastics (MPs; < 1 mm), can potentially affect a wide range of marine organisms. The effects on fish species, in particular species for human consumption, are still under debate. Few species have shown toxicological or physical impact, such as liver toxicity caused by the ingestion of virgin and/or contaminated MPs or the alteration of intestinal tissues. The European seabass (*Dicentrarchus labrax*) is one of the most consumed fish species in Europe and it is potentially exposed to the ingestion of MPs both in its natural habitat and in the aquaculture plants. We measured, by quantitative Real-Time PCR, the variation of four different early warning signals in the liver of the European sea bass exposed for 90 days to virgin (MPV) and marine polluted PVC (MPI) MPs supplemented with food. The selected genes are: the TNF receptor associated factor 3 (TRAF3), related to the activation of the immune and tumor-associated responses; the Peroxisome Proliferators Activator Receptors alpha and gamma (PPARα/γ), which mediate the responses to several chemicals including plasticizers; the Estrogen Receptor alpha (ERα), a nuclear receptor involved in the activation of transcription of estrogen-related molecular pathways, included xenoestrogens. The mRNA levels were quantified on 42 fish sampled at time 0, after 30, 60 and 90 days of exposure to the two treatments (MPV and MPI) plus control (CTRL). The expression of TRAF3 appears to be down-regulated with increasing time of exposure. The ERα mRNA levels are higher in the control of each treatment in comparison to MPI and MPV for all the exposures, suggesting an upregulation of the gene related to contaminated food pellets. On the contrary, the PPARα gene expression increases over the time from 60 to 90 days of exposure. The PPARγ does not show a temporal trend, but seems to be mostly affected by the MPV exposure, suggesting an effect due to leaching of plastic additives from PVC. The present study represents one of the first investigation on the effects of the exposure to virgin and marine polluted PVC MPs on an edible species, the European seabass. These results represent an early warning signal on the chemical and physical hepatic stress on this species. Further investigations are needed to better understand the role of the partitioning of chemicals from and to MPs, the physical effects of MP ingestion and the related effects on fish and, potentially, on human health.

Increasing the relevance of toxicity assessment in LCA: in the need for a cross fertilization between RA and LCA (I)

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Un-characterized elementary flows: to which extent is the quantification of the toxicity impact categories in LCA complete?
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The total number of elementary flows contributing to aquatic freshwater and human toxicity are in a range of 1500 to 2000, but the most common methods used for impact assessment associate a characterisation factor (CF) only a to small portion of them. This means that only a small fraction of the substances in the inventory, and usually a small mass, contributes to the overall impact score. A deep analysis of the life cycle inventory of 17 food products, taken as a case study, showed that the proportion of un-characterized flow can be very important: 55% of ELFLs. to air compartment (66% as mass), 32% of ELFLs. to soil (12% as mass) and 83% of ELFLs to water (24% as mass). This high number of un-characterized flows may lead to high underestimation of the toxicity impact categories and wrong identification of the hotspots. A check of the inventoried elementary flows that are not characterized at the LCIA phase should always be done to support interpretation of results. Indeed, the characterisation of the inventory into potential environmental impacts may not occur if some of the elementary flows are not covered by the chosen characterization models. Toxicity impact categories in LCA and in the Product Environmental Footprint (PEF) are often the dominating impact (after normalization) and metals are often identified as the most contributing elementary flows (ELFLs).

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Getting the chemicals right: Gaps and opportunities in addressing inorganics in life cycle assessment
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Life cycle assessment (LCA) is used to compare products and product systems in terms of their environmental sustainability and for that LCA needs to include all potential impacts on humans and the environment. Currently, quantifying the toxicity potential of several thousand organic substances and certain cationic metals is included in existing characterization models within life cycle impact assessment (LCIA). However, a variety of additional inorganic substances used e.g. in the textile, personal care, and building and construction industry are included neither in current life cycle inventory databases, nor current LCIA methods. Without the integration of the various economically relevant and potentially human toxic and/or ecotoxic inorganic substances such as inorganic salts, acids, bases and elements, however, no satisfying conclusions regarding the environmental sustainability of any technology containing any of these substances can be drawn. We provide an overview of different substance groups already incorporated in LCIA toxicity characterization modeling, the economic and environmental relevance of inorganic chemicals, and an outline of possible ways towards incorporating inorganic chemicals in LCIA toxicity characterization. The analysis of existing LCIA approaches of specific organic and inorganic chemical groups including PFASs, nanoparticles, salts causing salinization, and common ionic liquids show that the fate, exposure and effect modeling have to be adapted at various levels for the characterization of inorganic substances other than cationic metals. Differences in physicochemical properties and environmental fate and transformation processes of these specific substance groups compared to inorganic substance groups show that the existing LCIA model USEtox cannot be applied to inorganic substances without further modification towards including specific reaction- and process-kinetics. Possibly relevant chemical reaction pathways will be outlined as a necessary step toward improving the environmental fate and (human and ecosystem) exposure assessment of various inorganic substances. Also, we present an overview of the availability of ecotoxicity and human toxicity effect data for elements and inorganic compounds in state-of-the-art databases. An overview of how conventional toxicity effect data can be adapted and used for estimating toxicity-related effects of inorganic substances on humans and ecosystems will be provided.

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A trophic chain-based approach for ranking chemicals in LCA

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The intensive use of chemicals in agricultural and industrial systems may lead to potential negative effects on natural ecosystem and biodiversity. Increasingly, there is the need of orienting research efforts towards the improvement of the existing models and indicators for assessing chemical impacts along the supply chains, e.g. in the context of life cycle assessment (LCA) as well as for ecological risk assessment (ERA). As a result, prioritizing chemical substances according to their ecological relevance is becoming a major challenge for better characterising impacts of chemical emissions on ecosystems. In the present study we explore options for characterising chemicals, calculating effect factors (EFs) for species representing different components of an aquatic trophic chain. Those effect factors could be, then, applied in models, such as USEtox, for ranking chemicals. The study aims at presenting the methodology and discusing the potential added value of using few species. Current results are to be considered for illustrative pourpose only.

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Accounting for metal speciation in terrestrial ecotoxicity: a compromise between coherence across metals and validation data availability

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Metals often dominate terrestrial ecotoxicity impacts in LCA because models developed for organic compounds are directly applied to metals, raising a LCA credibility issue. In line with the Clearwater Consensus and freshwater ecotoxicity latest advances, new terrestrial ecotoxicity characterization factors (CFs) that include metal speciation were calculated and validated with field data for Zn using speciation model WHAM. New CFs were tested in a case study (production and use of 1 kWh in the Quebec region) and helped reduce the global terrestrial ecotoxicity impact score by around 25%. Considering that 75% of this score is related to metal emissions in soil, including speciation of other metals could modify considerably LCA results. The goal of this project is to determine the generalization potential of this method to other metals. New CFs include a fate factor calculated using USEtox with soil specific k_d values, a BF defined as the ratio of the soluble metal concentration on the total metal concentration in soil and an effect factor obtained with the assessment of the mean impact (AMI) method and available terrestrial ecotoxicity data. Soluble metal concentration and k_d values are calculated with speciation model WHAM 7.0 and with soil data from the Harmonized World Soil Database 1.21. For WHAM 7.0 validation, a literature review is undertaken to gather field data for metals listed in IMPACT2002 and/or USEtox. For each field soil sample, two BFs (BF_{exp} based on field data and BF_{WHAM} based on modeling) are calculated and compared (value-to-value and ranking). A difference of 2 orders of magnitude (the acceptable level of uncertainty for organic chemicals in LCA) between the 2 BFs is considered satisfactorily. The literature review allowed gathering small (20-53 samples) but varied field soil datasets for copper (Cu), nickel (Ni), cadmium(Cd), lead and silver and smaller sets (6-8) for manganese and cobalt , many of which are among the greatest contributors to terrestrial ecotoxicity in the kwh-case study. Results indicate so far that the 2 orders of magnitude criterion is validated for Zn, Cu, Ni and Cd. The spread of calculated world soil BFs for the various metals (4-5 orders of magnitude) highlight the need to regionalize metal terrestrial ecotoxicity CFs. WHAM seems a promising avenue to estimate speciation in this context. This approach should be considered for generalization in LCA but with care, as it can only be validated so far for 7 metals.

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Rooting LCA methods in experts' knowledge: Human cost of pesticides caused by agricultural practices

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Ecosystems and people are exposed every day to multiple chemical stressors via multiple pathways and routes due to economic and population growth. HERA and ELCA face many issues to account for the effects of all kinds of chemicals. The authors are developing a method to examine the comparative effects of different agricultural production systems on operators health because of pesticide handling. The objective is to enable the assessment of a comprehensive set of information for decision support purposes, taking into account the actual (good and bad) work practices. In order to build a method able to discriminate between several production systems regarding their impacts on operators health, we followed four steps: seeking help of experts; constructing knowledge trees; developing decision trees; calculating the human cost of pesticides for operators at different scales, and regarding one given farming system. The first phase was implemented through a Delphi expert consensus method eliciting ideas from agronomists, economists and exposure assessment specialists, in order to map the different “banana workflows” and the origins of good and bad practices. We suggest estimating the pesticide human cost for the “average operator” by adding the pesticide human costs of the *actions* in which he/she is involved. The *pesticide human cost* of one action is directly proportional to: the number of operators carrying out the action; the number of occurrence of the action; the degree of exposure of the “average operator”; the toxicity of the pesticide at stake (through the AOEL). Interpretation of the results of pesticide human cost calculations should be done only by comparing two or more production systems involving the same temporal and spatial scales. Indeed, the result of one given calculation in the absolute is meaningless. Currently, this method has been developed for banana production systems only, but its principles are suitable also to other crops. Meanwhile, we are developing knowledge trees for other agricultural workers than operators. We suggest that it is possible to root LCA methods in experts' knowledge, at least for target classes of chemicals, and for target specific populations.

Development and validation of standardised methods and their use in regulatory frameworks

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Systematic reviews - the missing link between the advancements of science and confident evaluation of environmental effects of chemicals

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Alternative approaches to use of the vertebrate animals in ecotoxicology are needed to support environmental hazard and risk assessments of chemicals under the ethical, legal, financial and public pressure in Europe. On the positive side, major advancements in our understanding of mechanisms of toxicity of different species have resulted in a great number of technologies that can now directly measure the biological events underlying (eco)toxicity. However, less progress has been made in agreement and standardization of these new tests, and their acceptance by the international regulatory authorities, without which the transition from the vertebrate animal-based tests to alternative models will be slow. There exists a disconnect between the advancements in the toxicological test methods and the decision-making in the regulatory arena. This missing link is Evidence-Based (EB) methodologies and specifically their principal tool, a systematic review (SR). SR methodologies have been developed in medicine and have led to vast improvements in the quality, transparency and consistency of clinical trials over the last few decades. EB methodologies are set out to assemble, assess, integrate, analyze and summarize the published literature in a transparent, objective, and consistent manner. This approach holds great promise for comparison of different test methods, as well as for chemicals risk assessment performed using these tests, and to become a standard that informs confident regulatory decisions. The theory and principles of evidence-based methodologies will be described and an example of the application of this approach as a systematic review of zebrafish embryotoxicity test method will be presented.

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Species Sensitivity Distributions with Censored Values

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Species Sensitivity Distributions (SSDs) are increasingly requested by regulatory authorities, especially in the EU, as part of product registration submissions. There is some inconsistency among country regulators and EFSA guidance as to requirements and acceptable methods of fitting and interpreting SSDs. The primary issue to be discussed the handling of censored values (e.g., EC50>100 ppm). It has become common in some EU registrations to reject species data for SSD purposes if the data do not conform to the log-normal distribution. There is no scientific basis for this requirement and its use does not serve regulatory conservatism. Indeed, exclusion or improper treatment of censored values tends to increase HC5 estimates. Another issue is forcing all data to fit a single distribution, such as log-normal. It is shown that this too can produce significant bias. The treatment of censored data in SSD fitting has not received as much attention as it deserves, given that right-censored data is common in toxicity studies and occasionally, left-censored values are found. The later are common in monitoring data, but not common in toxicity studies. Some recent regulatory guidance suggests discarding censored values in fitting an SSD. (e.g., EFSA 2014; EFSA 2015; Schmitz *et al.* 2015) or using them only under strict conditions (EFSAS 2013). There is no scientific basis for avoiding censored values or for treating them as uncensored. There have long been known mathematically correct ways to include censored values in fitting a distribution. Ignoring censored values means the distribution being analyzed is truncated and there are well known differences between truncated log-normal and log-normal distributions. The bias introduced by eliminating censored values will be demonstrated, both through datasets used in product registrations, computer simulations, and mathematics. A well-known mathematically correct approach to including censored values will be described. Force fitting a standard distribution or rejecting data that do not conform to this distribution, and exclusion or improper treatment of censored data will bias the HC5 and HC5LB estimates. Mathematically sound and tractable methods for selecting distributions and handling censored values are known and available in validated software. There is no good reason for ignoring sound science in fitting or interpreting SSDs.

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Ecotoxicological assessment of corrosion protection products used on hydraulic steel structures
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Corrosion protection is applied to extend the lifetime and function of steel components. In our study we conducted market research to establish which corrosion protection products are used in Switzerland. It appeared that epoxy resins have ca. 50% market share. Subsequently, we ran laboratory leaching tests with four products and determined the ecotoxicity of leachates using a suite of bioassays. The bioassays we ran were mainly (draft) ISO standards and one non-standard bioassay (the combined algae test) which has been applied in Switzerland in a regulatory context. However, currently no uniform or internationally adopted scheme is available to evaluate results from bioassays run on leachates from construction materials. For this reason we used a German scheme developed by DIBt (German Centre of Competence for Construction) to evaluate our bioassay results. In future, a scheme may become available through CEN (European Committee for Standardisation). CEN is currently preparing a report that will highlight the potential of bioassays for the evaluation of leachates from construction materials. Four types of epoxy resin based products were obtained and prepared according to instructions of the producers. Resins were directly applied onto glass plates without any primer. Two leaching experiments were performed as 7-day horizontal shaking tests using a volume to surface ratio of 10 L/m^2. Toxicity was observed in all tests and particularly two products showed elevated toxicity. For Product 1, a >1000-fold dilution was required to reduce bacterial bioluminescence inhibition under 20% (a threshold specified in the DIBt scheme). Samples from Product 3 induced large effects in several receptor activation assays. Bisphenol A measurements in these samples clearly linked this compound to the observed endocrine effects. Although worst case scenarios were tested, i.e. short hardening times and low volume to surface ratios, the observed effects often require high leachate dilutions to reduce effect-levels below 20%. The DIBt evaluation scheme proved useful for the ecotoxicological evaluation of the leachates. However, a uniform and internationally standardised and adopted evaluation scheme – covering an extended effect panel – is desirable. *Acknowledgement* - The authors thank the Swiss Federal Office for the Environment (FOEN) for financial support and industry partners for providing their products for testing.

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Are current algae toxicity tests suitable for the evaluation of carbon-based nanomaterials?
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Existing standard (eco)toxicity test guidelines (TGs) have been found to exhibit significant limitations in the reliable assessment of manufactured nanomaterials (MNMs). These include the preparation of MNM dispersions, dispersion stability during exposure and interference with measurement techniques e.g. through the presence of MNMs and their aggregates. This has resulted in significant challenges in establishing a functional risk assessment framework for MNMs. Recently, international efforts have been undertaken to address these MNM-related challenges and propose modified TGs. In this study, we evaluated existing standard TGs and modified TGs for freshwater algae ecotoxicity assessment using two carbon-based nanomaterials (CNMs); multi-walled carbon nanotubes (MWCNT) and graphene oxide (GO). Interactions of MWCNT and GO with chlorophyll-a (Chl-a) quantification were studied using (i) OECD 201 TG with *in vivo* Chl-a determination, (ii) OECD 201 TG with a modified *in vitro* Chl-a determination and (iii) ISO 10260 TG with *in vitro* Chl-a determination. The tested CNMs caused a high level of interference in *in vivo* Chl-a determination, resulting from shading of Chl-a fluorescence by CNMs and their aggregates (MWCNTs). Furthermore, GO caused a high background noise through autofluorescence and MWCNTs exhibited algal adsorption. Direct signal interference and autofluorescence were found to be sufficiently reduced using *in vitro* Chl-a determination approaches. However, both CNMs caused a concentration dependent loss of fluorescence signal, even when algae were extracted directly after CNM exposure. Furthermore, we also found a significant reduction of Chl-a fluorescence when MNMs were added after the extraction process, or shortly before fluorescence measurements. This indicates that the CNMs used in this study directly reduce the amount of free Chl-a. This likely due to adsorption processes as CNM feature high surface areas and strong adsorptive properties. While both materials significantly reduced the amount of measured Chl-a, the more hydrophobic MWCNTs exhibited stronger adsorption of Chl-a compared to GO. Owing to their specific physicochemical properties, toxicity assessment of CNM with algae remains challenging and this study emphasizes the need for further research towards the development and establishment of standard TG which are suitable for all MNM types.

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Poster spotlight: TH232, TH233

Global Horizon Scanning Project - open session

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Introduction to the programme and questions on vulnerability, propagation of effects, multiple stressors and effect modelling
P. van den Brink, Alterra and Wageningen University

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Research needs for high-throughput assessment of individual contaminants and their mixtures, both historical and emerging, and sustainable molecular design and alternatives analysis of chemical products
B.W. Brooks, Baylor University / Department of Environmental Science

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Prioritisation and detection of existing and emerging environmental contaminants
A. Boxall, University of York / Environment Department

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Predicting stressor effects on ecosystems and their services in a changing world: implications for risk communication, risk management and restoration
L. Maltby, The University of Sheffield / Dpt. of Animal & Plant Sciences

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Discussion on way forward

Cost effective and ecological relevant testing using invertebrate species: new insights for environmental risk assessment (II)

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The effects of DEHP, a potential endocrine disruptor, on an insect, the Egyptian cotton leafworm
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Di-(2-ethylhexyl)-phthalate (DEHP)[1] is a plasticizer widely used to increase plastic flexibility and known to have endocrine disrupting effects on vertebrate species. In this project we focus on the effects of DEHP on a crop pest: the Egyptian cotton leafworm, which can be exposed to these pollutants in some ecosystems by water contamination or atmospheric deposition on plants. As EDCs are known to have major effects during early development in vertebrates and some invertebrates we first investigated the effects of DEHP on the post-embryonic development (length and number of larval instars, larval weight and food consumption) and the sex ratio of *S. littoralis*. We also focused on its effect on fertility and male sexual behaviour. Indeed, this crucial process for mating is under endocrine control in our species[2] and could be potentially disrupted by DEHP. Besides, we investigated the potential modifications in the hemolymphatic ecdysteroid titration in larvae and adult males, using Enzyme Immuno Assay (EIA)[3]. For both those experiments, we chose to feed larvae, from the end of the 3rd larval instar to the last larval instar with either food with ethanol (control) or contaminated food at several concentrations of DEHP (from 10pg to 40mg DEHP per gram of food). We show that DEHP increases mortality, the length of larval instar and the weight of the larvae for the two highest concentrations (5mg/g and 40mg/g). An increase of larval hemolymphatic ecdysteroid titers at day 6 of the last larval instar was also observed at the lowest tested dose (10pg/g). Experiments are still in progress to investigate the mechanism of action of this chemical and the possible effect on adults. Actually, investigations on DEHP effects at different life stages are necessary to understand its putative effects on this insect pest, both at the individual and populational level. [1] ECHA - European Chemical Agency. 2008. European Union Risk Assessment Report –

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Biochemical and transcriptional responses in the freshwater crayfish Procambarus clarkii exposed to pharmaceutical mixture.
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Detection of pharmaceuticals in the aquatic environment is an emerging issue of concern. Although the majority of drugs occur in the aquatic environment at trace level (ng L^{-1} to low µg L^{-1}), most of them have been specifically designed to be biologically active at low concentrations in humans and animals and therefore they can represent a risk for aquatic organisms particularly under chronic exposure. The knowledge on the biological effects of pharmaceuticals on aquatic organisms is still scarce, moreover since these compounds occur in aquatic environment as complex mixtures. Ibuprofen (IBU) is one of the most used non-steroidal anti-inflammatory drugs: its ability to induce toxic effects in aquatic organisms at environmentally relevant concentrations has been widely proven. Ciprofloxacin (CIP) and flumequine (FL) are broad-spectrum antibiotics of the fluoroquinolones class. Fluoroquinolones toxicity was observed in rodents producing among others oxidative stress, cyto and neurotoxicity. However, ecotoxicological effects on aquatic organisms of CIP and FL are practically unknown. In our work biochemical responses related to oxidative stress (CAT, SOD, GR, T-GPx activities and LPO levels), detoxification (GST activity) and neurotoxicity (AchE activity) were studied in the red swamp crayfish *Procamabarus clarkii* after sub-chronic exposure to 10 and 100 µg L^{-1} of a mixture of IBU, CIP and FL. Recovery of organisms was also evaluated after 1 week of post-exposure depuration. Additionally transcriptional responses related to antioxidant and stress (*CAT*, *GST*, heat shock protein 90 *HSP90* and metallothioneins *MT*), apoptotic (cathepsin-L *CatL*, 14-3-3 zeta protein *14-3-3ζ*), immune (thymosin *Thy*, toll-like receptors *PcToll*) and inflammatory (collagen alpha chain-like protein *ColA*) processes and neurotransmission modulation (neuropeptide precursor protein *NP*, myosuppressin-like neuropeptide precursor *MSNP*, synaptome-associated protein *SNAP*) were evaluated. Results obtained for biomarkers and gene expression indicate a general activation of different oxidative stress related features in both enzymatic and transcriptional responses, particularly at the highest concentration of drugs. Therefore, exposure of pharmaceuticals mixtures to *P. clarkii* can produce oxidative stress as indicated by enzymatic and transcriptional responses. It has still to be evaluated if these effects can have long term physiological effects on aquatic ecosystems and human health

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BDE-47 induces oxidative stress, activates MAPK signalling pathway, and elevates de novo lipogenesis in the copepod Paracyclopina nana
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Brominated flame retardant, 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47), has received grave concerns as a persistent organic pollutant, which is toxic to marine organisms, and a suspected link to endocrine abnormalities. Despite the wide distribution in the marine ecosystem, very little is known about the toxic impairments on marine organisms, particularly on invertebrates. Thus, we examined the adverse effects of BDE-47 on life history trait (development), oxidative markers, fatty acid composition, and lipid accumulation in response to BDE-47-induced stress in the marine copepod *Paracyclopina nana*. Also, activation level of mitogen-activated protein kinase (MAPK) signalling pathways along with the gene expression profile of *de novo* lipogenesis (DNL) pathways were addressed. As a result, BDE-47 induced oxidative stress (e.g. reactive oxygen species, ROS) mediated activation of extracellular signal-regulated kinase (ERK) and c-Jun-N-terminal kinase (JNK) signalling cascades in MAPK pathways. Activated MAPK pathways, in turn, induced signal molecules that bind with the transcription factors (TFs) responsible for lipogenesis to *EcR*, *SREBP*, *ChREBP*promoters. Also, the stress stimulated the conversion of saturated fatty acids (SFAs) to polyunsaturated fatty acids (PUFAs), a preparedness of the organism to adapt the observed stress, which could be correlated with the elongase and desaturase gene (e.g. *ELO3*, *?5-DES*, *?9-DES*) expressions, and then extended to the delayed early post-embryonic development and increased accumulation of lipid droplets in *P. nana*. This study will provide a better understanding of how BDE-47 effects on marine invertebrates particularly on the copepods, an important link in the marine food chain.

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Mechanisms of action of compounds that enhance storage lipid accumulation in Daphnia magna.
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Accumulation of storage lipids in the crustacean *Daphnia magna* can be altered by a number of exogenous and endogenous compounds, like 20- hydroxyecdysone (natural ligand of the ecdysone receptor, EcR), methyl farnesoate, pyrirproxyfen (agonists of the methyl farnesoate receptor, MfR) and tributyltin (agonist of the retinoid X acid receptor, RXR). This effect, analogous to the obesogenic disruption in mammals, alters Daphnia's growth and reproductive investment. Here we propose that storage lipid accumulation in droplets is regulated in Daphnia by the interaction between the nuclear receptor heterodimer EcR:RXR and MfR. The model was tested by determining changes in storage lipid accumulation and on gene transcription in animals exposed to different effectors of RXR, EcR and MfR signaling pathways, either individually or in combination. RXR, EcR and MfR agonists increased storage lipid accumulation, whereas fenarimol and testosterone (reported inhibitors of ecdysteroid synthesis and an EcR antagonist, respectively) decreased it. Joint effects of mixtures with fenarimol, testosterone and ecdysone were antagonistic, mixtures of juvenoids showed additive effects following a concentration addition model, and combinations of tributyltin with juvenoids resulted in greater than additive effects. Co-exposures of ecdysone with juvenoids resulted in de-regulation of ecdysone- and farnesoid-regulated genes, accordingly with the observed changes in lipid accumulation These results indicate the requirement of ecdysone binding to the EcR:RXR: MfR complex to regulate lipid storage, and that an excess of ecdysone disrupts the whole process, probably by triggering negative feedback mechanisms.

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D-counter: Automatic Organism Counting and Characterization in Ecotoxicology assays
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The chronic toxicity test with the microcrustacean *Daphnia magna* is one of the most commonly used tests in aquatic Ecotoxicology. This test requires the counting of the number of offspring produced by daphnids during 21 days. Currently, the counting is performed by technicians, manually, in backlight conditions. However, the counting procedure is not only time consuming, but also very susceptible to human error and even to visual impairment of the technician. Therefore, the development of a device for automatic counting is of sum importance. Following this line of thought, we present a device for automatic counting of *Daphnia* offspring, which can also estimate *Daphnia* body length – the D-counter. The device forces the passage of the organisms through a group of optical sensors. The acquired digital signals are then processed in real-time by a computing system in order to automatically count and characterize each organism. To validate the estimation of the daphnids BL a short test was carried out using daphnids with varying sizes (0-21d old) (three replicates per age). Each daphnid passed through the D-counter 3 times. The daphnids BL was measured under a stereomicroscope, and compared to the values obtained by the D-counter. Considering the estimation of daphnids BL, the values obtained by the D-Counter correlate well with the daphnids BL measured under a stereomicroscope (linear regression n=33, r^2-adjusted=0.900, F= 289.6996, p< 0.0001). Regarding the life table experiment, no significant differences were observed between the counts of both technicians (Mann-Whitney U Statistic= 6726.000, T=13512.000, n=136, p=0.998). For this reason, these counts were averaged and assumed as the manual counting data set. In parallel, the number of offspring obtained using D-counter device was similar to the manual counting data set, as illustrated in Figure 3 (Mann-Whitney U Statistic= 6568.000, T= 13674.000, n= 136, p= 0.755). The obtained regression (n=136, r^2-adjusted=0.920, F= 1588.1205, p< 0.0001), corroborates that manual and D-count counts are not statistically different. Overall results show the lack of significant differences between the manual and the D-counter estimated values concerning both BL and number of offspring. The D-counter device is a useful tool to be applied in ecotoxicology assays involving *Daphnia* sp, or other aquatic organisms such as *Arthemia* sp. Preliminary tests also showed its application in *Danio renio* eggs differentiation and counting.

Fish model species in human and environmental toxicology (II)

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Toxicity and neurotoxicity profiling of European sediments samples with Danio rerio embryos: A novel multi endpoints assay
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The biological evaluation of environmental samples is a crucial step to estimate potential risks for ecosystems and human health. However, chemicals are present in mixtures in the environment resulting in unpredictable outcomes due to complex interactions. Sediments are as well sinks for hydrophobic persistent organic pollutants such as PAHs, PCBs or brominated flame retardants. Recently, it was also suggested that more polar compounds could accumulate in sediments and significantly contribute to sediment toxicity. Moreover, the neurotoxic potential of sediments is still not well described in literature. In this study we propose a 96 hours test with multiple toxicological endpoints for sediments toxicity characterization using Danio rerio embryos. We selected six different endpoints focusing on neurotoxicity. Danio rerio embryos is a cheap and high-throughput model for developmental neurotoxicity and it could be used to rapidly screen environmental contaminants for potential neurotoxicity prior to extensive mammalian evaluation [3]. Extracts of native sediments from 10 spots (7 different European river basins plus 3 reference spots) were loaded on silicon O-rings at concentrations resembling the concentration in sediment organic matter for exposure of the embryos and the effects were observed for several toxicity end-points: spontaneous movements, edema, lethality, deformations, AChE activity and locomotor response.

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Zebrafish as a Sensitive Model for Assessment of Neurotoxic Effects of Nanoplastics
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Concurrent with the increasing pollution of microplastics and nanoplastics in the aquatic environment, comes a growing concern of these small-sized plastics, which are threatening aquatic organisms via diverse toxic mechanisms that are, at present, poorly understood. The present study investigated the toxic effects of nanoplastics toward zebrafish (*Danio rerio*) larvae and adults. Zebrafish were exposed to either nanoplastics alone, or the mixture of nanoplastics and a representative endocrine disruptor chemical. As for zebrafish larvae, treatment with nanoplastics alone inhibited the larval locomotion. Moreover, nanoplastics showed a synergic disruptive effect for 17α-ethynylestradiol (EE2) on the swimming hypoactivity phenomenon in the co-exposure treatments. Furthermore, four principal parameters which affected the larvae's behavior were screened out, namely oxidative stress ($p < 0.05$), body length ($p < 0.05$), nervous system related genes ($p = 0.11$), and visual system related genes ($p = 0.72$).However, as for zebrafish adults, the oxidative damage and body length alteration have not been detected in nanoplastics alone, nor in the co-exposure groups. Nevertheless, the nanoplastics still induced neurotoxicity, which may be due to the changes in the cholinergic system, impact of dopaminergic signaling and the development of the neuron. These results suggest that nanoplastics can function as a carrier to increase the neurotoxicity to zebrafish and aquatic organisms will have different toxic outcomes if they were exposure at different growth periods. This study can serve as a base for future investigations of detailed pathways of the toxic mechanisms of nanoplastics and will hopefully increase awareness of the potential environmental risks of nanoplastics.

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Toxicity of bifenthrin during early zebrafish (Danio rerio) development
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The pyrethroid insecticide bifenthrin has been increasingly employed for pest control in urban and agricultural areas in the United States of America over the last decades. Exposures to nanomolar (nM) concentrations of bifenthrin have led to altered calcium oscillations in rodent neurons. Ryanodine receptor (RyR) signalling is an important component of many calcium-dependent signalling cascades, including the mTOR signalling pathway. RyR activity and mTOR signalling are critical in neurodevelopmental processes that determine the patterns of neuronal connections laid down in the developing brain, such as dendritic outgrowth and synaptogenesis. Our main objectives in this study were to examine transcriptomic responses to nM concentrations of bifenthrin, focusing on genes in RyR and mTOR signalling pathways, in association with behavioural evaluation in zebrafish (Danio rerio). Wild type zebrafish were exposed for five days to 1, 10 and 50 ng/L bifenthrin, followed by a two week recovery period. Assessments were conducted at 1, 3 and 5 days post fertilization (dpf), and at 19 dpf. Endpoints included transcript analysis, locomotor behaviour, swimming performance and response to a predator cue. Bifenthrin exposures caused significant concentration-dependent effects on the majority of genes examined in both signalling cascades at all time points, including the end of the recovery period. Locomotor behaviour and predator response confirmed altered activity with main effects observed in animals exposed to the two lowest concentrations, suggesting altered neurological performance. However, differences in swimming performance were not observed as a consequence of bifenthrin exposure. These findings illustrate significant influences on neurodevelopmental processes in larval zebrafish exposed to nM concentrations of bifenthrin during early development. Alterations in behaviour became most evident after the recovery period, suggesting delayed and long-term effects of developmental exposures to this pyrethroid insecticide.

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Bioaccumulation and biological effects of tritiated water using zebrafish (Danio rerio) embryo-larvae model
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Tritium (^3H) is the radioactive isotope of hydrogen. It decay by emitting a low-energy electron with a short range and a high ionization density. Thus, ^3H energy deposition is localized and can be considered negligible via external exposition. However, ^3H uptake can be a threat particularly to DNA, an ionizing radiation target. ^3H is released in aquatic systems as tritiated water (HTO) by nuclear power plants and reprocessing plants. Futur use of nuclear fusion (ITER) is expected to increase HTO levels in ecosystems. This work aimes at evaluating the toxic action modes of HTO on the well-described zebrafish (*Danio rerio*) embryo-larvae model. To reach this objective, zebrafish eggs were exposed at two HTO concentrations ($1.22.10^5$ and $1.22.10^6$ Bq.mL^{-1}); fish were sampled at egg stage and larva stage. The first step aimed at assessing HTO bioaccumulation to characterize as accurately as possible the dose absorbed by organisms and then assess dose-response relationships. The second step focused on biological effects (developmental endpoints, Transmission Electron Microscopy (TEM), DNA damages and transcriptomics analysis). After exposure to both concentrations, ^3H activity was higher in eggs than in larvae. Comparison between concentrations showed that ^3H activity was 5 times greater for the highest concentration for both stages. Moreover, the calculation of concentration ratio (CR) showed that the equilibrium organisms-medium was not reached for both stages. Despite the exposure duration, CRs were the same between stages for the two concentrations. However, the comparison between CRs for both stages at the two concentrations show that CRs of eggs are differents, as are those of the larvae. Futhermore, there was an increase of DNA damages for both stages for the lowest concentration. No differences was highlighted in DNA damages for the highest concentration, which could suggesting a possible onset of repair mechanisms. For both concentrations, TEM analysis showed some muscle alterations in larvae but, neither mortality nor hatchability were affected. These results will be completed by the transcriptomics and γ-H2AX analysis. Regarding our first results, it looks like the kinetics of HTO bioaccumulation may vary according to stage and medium concentration. Moreover, it seems that DNA damages and repair depend on a concentration threshold. With this work we want to better understand the threat of ^3H on zebrafish embryo-larvae and, by extent, to the environment.

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Poster spotlight: TH035, TH036, TH051, TH053

Determining population relevance of ecotoxicological effects

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The 'so what' question: when is an effect actually an effect?
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Wildlife risk assessments for plant protection products (PPPs), assessed under Regulation (EC) 1107/2009, are based on data from acute and chronic toxicity tests. With risk assessment schemes becoming more and more complex, risk assessments are increasingly reliant on higher-tier data in order to demonstrate acceptable risks. The basic premise is to compare the response of organisms in an untreated control with those organisms exposed to a PPP treatment and determine an appropriate endpoint depending on the extent of adverse effects observed. Typically, statistical tests are used to help differentiate such effects, with a probability of less than 1 in 20 (p< 0.05) that the response observed will occur due to chance alone being the usual criterion. However, just because an 'effect' is statistically significant doesn't mean it is biologically significant; a number of factors should be considered in addition to the outcome of the statistical analysis. These include the magnitude of the difference between control and PPP treatments (e.g. would a 5% reduction in offspring weight have a significant impact on wild mammal populations?); the sample sizes in the control and PPP treatments (e.g. if a statistically significant difference is based on 5 individuals of species X in the control replicates and 2 individuals of species X in the PPP treated replicates in a mesocosm study, is this of consequence for wild populations of species X?); the duration of the effect (e.g. if a statistically significant difference occurs on only a single day mid-way through a study, would this significantly impact on wild populations?); and is a statistically significant difference occurring only in an intermediate dose due to chance rather

than a treatment-related effect? The aim of this poster is to present case studies that highlight the importance of evaluating the biological relevance of differences between treatments to determine whether statistically significant differences are likely to be relevant for wild populations.

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Population-Relevant Endpoints in Ecotoxicological Hazard and Risk Assessment Endocrine-active Substances (EAS)

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For ecological risk assessment, endocrine disrupters require the establishment of an endocrine mode of action (MoA) with a plausible linkage to a population-relevant adverse effect. Current ecotoxicity test methods mostly incorporate apical endpoints although some also include mechanistic endpoints, at the subcellular through organ level, which can help establish an endocrine MoA. However, the link between these endpoints and adverse population-level effects is often unclear, except for well-documented historical cases (*e.g.*, bird population declines and DDT; dogwhelk population declines and TBT). The presentation will be based upon case studies of endocrine-active substances (EAS) (tributyltin, ethinyl estradiol, perchlorate, trenbolone, propiconazole, and vinclozolin) that were used to evaluate the population relevance of toxicity endpoints in various taxa according to the OECD Conceptual Framework for Testing and Assessment of Endocrine Disrupters in the context of the the SETAC Pellston Workshop™: *Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals*. Tributyltin provides a well-documented case-study where population-relevance of endocrine disruption is well established for molluscs. For other groups and other EAS, however, the population relevance of observed effects is not as well understood. In most cases, the strength of the relationship between test endpoints and population-level effects is uncertain. Furthermore, testing alone is insufficient for assessing potential adaptation and recovery processes in exposed populations. For this purpose, the development of models that link effects observed in laboratory tests to the dynamics of wildlife populations requires a more robust knowledge of the factors regulating these dynamics. As our understanding of (i) endocrine perturbations and key-event relationships and (ii) environmental regulation of population dynamics improves, adverse population level effects should be more easily and accurately predicted.

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Secondary Sex Characteristics - How can changes be interpreted with respect to ED mediated adverse effects?

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To demonstrate a biologically plausible link between an endocrine mode of action and a possibly resulting adverse effect at the (sub) population level is a crucial step during the identification of endocrine disruptors according to the WHO definition. Apical adverse effects are derived from higher tier in vivo data. Endpoints analysed in these assays comprise the histological examination of the sex organs and/or the genetic sex determination. A shift in the sex ratio of the tested species is taken as a strong adverse effect impairing population stability. Since the histological and the genetic sex determination require some effort, data for regulatory purposes are limited. Much more data are available when looking at secondary sex charateristics as endpoints. Since they are easier accessible, they are frequently reported in screening tests and non-guideline academic studies. Furthermore, they are often found to be equally sensitive than gonadal histology. However, their determination shows some specific disadvantages like the difficulty to be quantified, lower statistical power, influence of other stressors and not all fish models show specific secondary sex characteristics. To use secondary sex characteristics to prove endocrine mediated adverse effects, the question what they can tell us about the population relevance following their alteration needs to be answered. To start a scientific debate on this issue some key questions are broad up and discussed. Are changes in secondary sex characteristics an adverse effect per se? Are some changes in secondary sex characteristics more adverse/indicative than others? Which weight of evidence must be given to accept changes in secondary sex characteristics as adverse? From a regulatory viewpoint it is highly eligible to give secondary sex endpoints more weight. From the scientific perspective these endpoints are linked to some difficulties that need to be discussed before coming to regulatory sound conclusions.

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Modelling breeding success in birds potentially exposed to treated seed

A. Lawrence, Cambridge Environmental Assessments / Regulatory Ecotoxicology; J. Crocker, H. Lyons, Independent Consultant

This poster will summarise UK HSE/DEFRA funded project PS2373 "Development of reproductive risk assessment methods for birds potentially exposed to treated seed". The current avian reproductive risk assessment procedures for plant protection products according to EC Regulation 1107/2009 are designed to preserve the surrogate protection goal of making any reproductive effects unlikely (EFSA, 2009). Conventional avian reproductive risk assessments for pesticide treated seeds have a relatively high 'failure' rate at Tier 1 (indication of potential risk), but potential conservatism in the risk assessment would suggest a proportion of these failures are 'false positives'. Potential conservatism includes coincident breeding and drilling activity. In addition, toxicity endpoints may relate to a breeding phase which may not be exposed in reality. We modelled the effects of seed treatments of spring crops on the reproductive success of 4 farmland bird species (rook, linnet, skylark, yellowhammer). We ran two types of model, a "broods-at-risk" model based solely on the nesting dates supplied by the British Trust for Ornithology which estimates only the proportion of those nests that suffered toxicity exposure ratios (TERs) less than 5; and a "seasonal success" Markov chain model which estimated the number of chicks successfully raised by a typical female in the course of a breeding season. We extended the second model, which described average individual reproductive success, to consider the potential effects of pesticide on populations and their long-term growth rates. In nearly all scenarios rooks were noticeably more ecologically sensitive to pesticide seed treatments than other bird species investigated. Rooks begin breeding earlier than the other species and are more likely to breed at a time when treated seeds are being sown. Temporal overlap was smallest for the yellowhammer and intermediate for linnets and skylarks. In all models, this is largely reflected in species vulnerability to pesticide effects with rooks faring worst, followed by skylark and linnet and with yellowhammer being least affected by seed dressings. It was demonstrated that individual reproductive TERs < 5 do not necessarily translate into complete breeding failure at the population level. Under some scenarios, individual reproductive TERs < 5 may translate into impacts on PGR of < 1% reduction compared to a control scenario, or less (including zero reduction).

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Methods for quantitative weight of evidence assessment of higher tier studies on the toxicity and risks of neonicotinoid insecticides in honeybees

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A quantitative weight of evidence (QWoE) methodology was developed and used to assess a number of higher-tier studies on the effects of three neonicotinoid insecticides clothianidin (CTD), imidacloprid (IMD) and thiamethoxam (TMX) on honeybees. The WoE methodology was used to characterize the quality of the available studies from the literature and unpublished reports conducted by the registrant to assess their relevance to potential or measured adverse effects. These higher tier-studies focussed on exposures of honeybees to neonicotinoids via several matrices as measured in the field as well as effects in experimentally controlled field studies. Reports provided by registrants and papers from the open literature were assessed in detail, using pre-defined criteria for quality and relevance to develop scores (on a relative scale of 0-4) to separate the higher quality from the lower quality studies and the relevant from the less-relevant results. The scores from the QWoEs were summarized graphically to illustrate the overall quality of the studies and their relevance. Through mean and standard errors, this method provided graphical and numerical indications of the quality and relevance of the responses observed in the studies and the uncertainty associated with these two metrics. All of this analysis was conducted transparently and the derivation of the scores were fully documented. Examples of the use of this procedure are provided with data from the assessments of IMD, CTD, and TMX

Advances in Exposure Modelling: Bridging the gap between research and application

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Modelling exposure to ionizing substances with spatial and temporal resolved models: A case study for multiple pharmaceuticals in the Baltic area

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Water resources contamination by pharmaceuticals is widespread with more than 600 pharmaceuticals detected in surface waters of 71 countries. Because several classes of pharmaceuticals are known to affect wildlife adversely, the European Medicines Agency (EMA) has set a guideline on environmental risk assessment (ERA) covering human pharmaceuticals. The process covers a two-tiered assessment with a modelling step for an environmental exposure scenario and a second step for testing environmental fate and effects. However, the recommended exposure modelling disregards spatial and temporal variability and uses simplistic relationships resulting in limited realism. STREAM-EU (Spatially and Temporally Resolved Exposure Assessment Model for European basins) is a model able to simulate spatially and temporally resolved environmental concentrations in river basins hence providing improved exposure assessments. Contrarily to classical models, STREAM-EU also provides mechanistic process-based modelling for a wide range of substances, including ionic organic substances, rendering the model applicable to simulate contamination by pharmaceuticals as most of them are weak acids or bases and thus ionized at environmental pH. In this study, a comprehensive exposure assessment for pharmaceuticals in Swedish surface waters was made using STREAM-EU model. Results indicate that Metformin ($27x10^3$ ton/y), Paracetamol ($6.9 x10^3$ ton/y) and Ibuprofen ($2.33 x10^3$ ton/y) were the drugs with higher amounts reaching the Baltic Sea in 2011. 35 of the studied substances had more than 1 ton/y of predicted flush to the sea. Exposure potential given by the ratio amount of the drug exported to the sea/amount emitted to the environment was higher than 50% for 7 drugs (Piperacillin, Lorazepam, Metformin, Hydroxycarbamide, Hydrochlorothiazide, Furosemide and Cetirizine), implying that a high proportion of them will reach the sea, and below 10% for 27 drugs, implying high catchment attenuation. The present study is the first to provide a comprehensive prediction of surface waters contamination by prescription drugs in Sweden. Given that Sweden has currently no systematic monitoring programme for pharmaceuticals, our results are extremely valuable as a basis for prioritization and risk assessment studies. At present, it is not possible to estimate the ecotoxicological risks associated with exposure of aquatic organisms to pharmaceuticals in Sweden because of data gaps for ecotoxicity endpoints.

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European environmental scenarios of chemical bioavailability in freshwater systems
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The goal of environmental risk assessment (ERA) is to quantify the risk that a given chemical would impair the structure and function of natural ecosystems by assessing its environmental exposure and the expected ecological aspects. However, the environmental realism, ecological relevance and methodological accuracy of the currently used exposure and effect assessment approaches have been questioned for years. The issue has been recently underlined in a joint opinion from the three scientific committees of the European Commission, in which the major challenges for risk assessment were devised. Concerning exposure assessment, the transition to more dynamic and realistic modelling approaches and scenarios was suggested, since it would allow a more accurate prediction of bioavailable concentrations and their variations in space and time. In this work, an improved dynamic multimedia model (ChimERA fate), including a phytoplankton compartment and equations to calculate phytoplankton, detritus and dissolved organic matter variations in time, was developed. The model was parameterized to simulate five dynamic scenarios for shallow meso-eutrophic phytoplankton-dominated water bodies based on a latitudinal gradient (in Europe); such scenarios include seasonal profiles of water temperature, autochthonous phytoplankton biomass, detritus, and dissolved organic matter. Model runs were performed for a number of chemicals with increasing hydrophobicity (8 PCBs), with the aim of investigating the influence of scenario characteristics and compound properties on bioavailable concentrations. The key processes were adsorption/uptake by phytoplankton and deposition to sediment of detritus-bound chemicals. The northern scenarios (“Scandinavia” and “UK”) showed the highest bioavailable concentrations, with max/min ratios up to 25; in contrast, for example, maximum concentrations in the “Mediterranean” scenario were lower by a factor of 2 to 9 with respect to the northern ones (depending on chemical hydrophobicity), due to the generally higher biomass and carbon levels, and showed only limited seasonal variability (up to a factor of 4). These results highlight the importance of including biomass and organic carbon dynamics in both modelling approaches and scenarios for the evaluation of exposure concentrations in aquatic environments.
Acknowledgement - The ChimERA project is financed by the Long-range Research Initiative of CEFIC (www.cefic-lri.org) (project code: LRI-ECO19).

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Evaluation of a global multiscale multimedia fate model framework applied to home and personal care products in Asia
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Typically, chemical exposure assessments are either conducted using course approaches at a low resolution, large scale (e.g. global) or with detailed processes and data at high resolution but limited scale (e.g. catchments). Here we present a geographically refined modelling framework to improve the environmental risk assessment of chemicals used in home and personal (HPC) products, combining spatial emissions (ScenAT) fate and elimination in sewage treatment plants (SimpleTreat) and the spatial multiscale multimedia fate model Pangea. We used this framework to study emissions associated with HPC products in 17 countries across Asia and Oceania. Spatial distributions of environmental concentrations for 16 relevant substances and were compared to results obtained with the LCA consensus model USEtox™ (non-spatial), and in a second step with 1600 georeferenced monitoring data for freshwater and sediments collected from the scientific literature. There is generally a good agreement between Pangea and USEtox results, and between Pangea results and monitored values in freshwater. This is not true for sediments, especially for parabens, for which the reported K_{oc} cannot explain the high observed sediment concentration and deserves further investigation. We discuss the challenges that arose in the comparison of large scale model with experimental data, learnings and plans for future developments, and demonstrates the use of the model to design future monitoring campaigns. \n

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SimpleBox approach to exposure modeling of nanomaterials - the next step!
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In order to bridge the gap in environmental exposure assessment between the scientific community and regulatory domain, tools are needed that can be used off the shelf. This is specifically important for exposure assessment of nanomaterials, where many publications report nanomaterial fate or exposure modelling aproaches, but these cannot be readily applied outside the scientific community. This was also the case for SimpleBox4nano as the first step was the development and testing of the model definition. This model definition has now been incorporated in the original SimpleBox tool and made available as a beta release on rivm.nl/simplebox. This means that now the nested spatial scenario can be used, which includes regional, continental and global scales and several sub-compartments, e.g. sea and fresh water compartments. Furthermore, the concentration of the dissolved species can be calculated in parallel to the particulate species of a chemical. However, the complexity of SimpleBox4.0-nano has increased in comparison with the original, with many more equations and input parameters required for nanomaterials. The next step would be the releas of the first final version of SimpleBox4.0-nano. To do this the tool needs to be tested and possibly adapted in order to fulfil the regulatory demands of a screening level exposure assessment for which SimpleBox4.0-nano is designed. Here we report on a test of one of the fundamental aspects of SimpleBox and other models, which is predicting steady state exposure concentrations for nanomaterials. This is done using a probabilistic aproach for calculating PECs for three case studies on TiO_2, Ag and C_{60} nanomaterials. The uncertainty in predicted PECs is mainly explained by the size of nanomaterials and natural particles (SPM) together with their attachment affinity. The time dynamic analysis showed that the PECs for large agglomerates of nPs and SPM is not reached within 1 million years in seawater and marine sediment. The results show that the current version of SimpleBox4.0-nano is fit for predicting exposure concentrations of nanomaterials. However, the currently used scenario might not be the most relevant from a regulatory perspective. Further work is needed on a more user-friendly method to find or estimate the required input parameters and creating a relevant model scenario.

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Can release rates be estimated well enough to allow 'inverse posteriorization' of REACH substances?
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In the EU project SOLUTIONS project, partners work together to foresee the emergence of new chemicals of concern. The partners do so by constructing and testing the so-called ‘SOLUTIONS modeling train’, by which ecological and human impacts of new chemicals are predicted from registered or estimated EU-wide market volumes and registered or estimated substance properties. The first ‘carriage’ of this train is release estimation: what proportion of the market volume finds its way to the environment? The SOLUTIONS project has adopted a first-principles estimation model for this purpose, based upon the spERC tables provided by industry. How reliable (i.e. accurate, precise) is this emission estimation model is for the purpose of predicting ‘impact’ to the environment? We have tested the emission estimation model by implementing it into a simple mass balance model as described in the REACH guidance for exposure assessment modeling, and combining this with the Van Straalen-Aldenberg integral for calculation of mixture toxic pressures in the aquatic environment, comparing the outcome of the model calculations with what is known by observation. We have chosen to focus on the possibility to predict the combined toxic pressure of complex mixtures of substances, as they occur in natural fresh waters, and reason how much individual chemicals contribute to this. We show in this paper that, although highly uncertain, output from the newly developed emision estimation model is accurate and precise enough to allow so-called ‘inverse posteriorization’, i.e. filtering out the few ‘least innocent’ substances by assessing the ‘most innocent’.\n We conclude that emissions of REACH substances can be estimated with sufficient accuracy and precision to allow (i) assessment of their combined toxic pressure on aquatic communities, and to allow (ii) assessment of the ‘least innocent’ 1% of chemicals. Toxic pressure estimation from market volumes may lead to overestimation of

exceedance of acute EC50 values, but not to an extent that the approach must be rejected as unrealistic. Further investigations of systematic overestimation of emissions are under way.

Organic micropollutants in the environment: analytical challenges and engineering innovations (IV)

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Characterization of surface- and wastewater samples using the planar Yeast Estrogen Screen (pYES)

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The planar Yeast Estrogen Screen (pYES) is a powerful approach for the detection and quantification of estrogenic compounds in surface and wastewater samples. It directly combines thin layer chromatography (TLC) with the Yeast Estrogen Screen by the application of the modified yeast cells directly on the surface of the TLC-plate after chromatographic separation. With this approach activity profiles of samples can be generated easily opening a wide range of different applications, e.g. source tracking of estrogenic compounds in the environment, comparative assessment of alternative processes for wastewater treatment and due to its high sensitivity a compliance check of the EU-WFD watch-list compounds E1, E2 and EE2. Amounts of 1 pg E2 and EE2 can be detected and quantified by the pYES. This allows the quantification of E2 and EE2 in the range of 10 pg/l after a 1000-fold concentration of the sample using solid phase extraction. These low limits of quantification are necessary for a reliable compliance check of EE2 with a discussed environmental quality standard of 30 pg/l. For a validation of the proposed method, results obtained by the pYES were compared to results of a LC/MS-HR measurement. Correlation coefficients for waste water and surface water samples were > 0.9 indicating a satisfying accordance between both methods. The results measured for the analytes E2 and EE2 were consistent as well. In addition to the chemical analysis the pYES as an effect based approach can detect structurally uncharacterized compounds sharing the same mode of action, i.e. the activation of the estrogen receptor. This possibility gives valuable information about compounds contributing to the overall effect of a given sample and highlights yet unidentified compounds which might be of concern because of their endocrine potential. Taken together, the pYES-approach is robust and sensitive and seems to have a high potential to be used as a fast screening tool for various applications. The approach is complementary to the more common combination of HPLC with a subsequent bioassay and classic chemical analysis.

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Breaking new ground in high-throughput analysis: MALDI-TOF MS for the quantification of explosives in contaminated soil

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Around the world vast areas of former production and testing sites for ordnance are still fenced off and not usable because of the contamination with nitroaromatic explosives. Particularly 2,4,6-trinitrotoluene (TNT) and its degradation products constitute environmental and health hazards and require risk assessment. Conventional analysis tools for samples from those areas such as HPLC and LC/GC-MS are time-consuming, not suited for high-throughput, and therefore not applicable for the spatial delimitation of contamination. Although it was originally not engineered for the detection and quantification of small molecules, MALDI-TOF mass spectrometry is a cheap, simple and fast method to analyse hundreds of samples in a very short time. Hence, we developed a MALDI-TOF MS method to detect and quantify TNT as well as its degradation and by-products in soil samples. A crucial point is the choice of the matrix. It is needed to ionize the analytes but not allowed to suppress the signals of target ions. Previously, 1,5-diaminonaphthalene (DAN) has shown good properties for the negative ionisation of different analytes. Thus, we successfully applied DAN as a matrix for the sensitive detection of TNT, aminodinitrotoluenes (ADNT) and dinitrotoluenes (DNT) as well as 1,3,5-trinitrobenzene (TNB) and 1,3-dinitrobenzene (DNB). Without a further separation technique it is not possible to distinguish isomers, but the total amount of ADNTs and DNTs can be determined. All standard substances showed signals of the radical anion as well as specific fragments. Principal component analysis (PCA) revealed the concentration dependency of those signals. Normalized calibration curves of the ion abundances were established using an internal standard, followed by the quantification of different extracts of highly and slightly contaminated soils. The MALDI-TOF MS quantification obtained similar results compared to the conventional HPLC analysis. Furthermore, the statistical computing project R including the web application R Shiny and the package *MALDIquant* were used to establish a fully automated analysis tool. Thus, MALDI-TOF MS measurements of calibration standards and samples as well as the quantification of explosives can be finished in less than an hour. In conclusion, we developed a fast, cheap and high-throughput MALDI-TOF MS method that can be a valuable screening tool for the spatial delimitation of explosives contaminated areas and exploited for further chemical stressors.\n

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Bioimaging of the Fungicide Fludioxonil in Formulation-Coated Wheat Seeds utilising ToF-SIMS

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ToF-SIMS has been increasingly widely used in recent years to look at biological matrices, in particular for biomedical research, although there is still a lot of development needed to maximise the value of this technique in life sciences research. The main issue for biological matrices is the complexity of the mass spectra and therefore the difficulty to specifically and precisely detect analytes in the biological sample. Here we explore the use of ToF-SIMS in the agrochemical field, which remains a largely unexplored area for this technique. In particular, we selected fludioxonil, a halogenated fungicide, as a model compound to test the approach. contained in a formulation used to coat wheat seeds. Fludioxonil is widely used, with $285 million global sales in 2014; one of its applications is as a seed coating, where it is applied to seeds before planting as a way of reducing losses to fungal infection. We then used a TOF-SIMS V (IONTOF) with a Bi_3^+ primary ion source for imaging fludioxonil in treated wheat seeds, in negative ion mode. One challenge to be overcome was sample preparation, as the seeds cannot easily be sectioned using standard cryosectioning approaches, as they are too dry and friable. We tested different embedding media to find the most suitable for ToF-SIMS analyses and for seed samples. In addition, we used an Ar secondary beam for sputtering the sample for depth profiling. We were able to detect and localise the fungicide in the coated wheat seeds, in actual treated commercial seed samples. Furthermore, it also gave useful complementary information on other chemicals present in the formulation. Future experiments will focus on application of ToF-SIMS, with high sensitivity and spatial resolution, to detect agrochemicals in biological matrices in general; e.g. including transfer between biological systems, such as from plants to pest species.

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The Story of TCPP Indoors and Outdoors: Sources, Concentrations and Fate

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Tris(chloropropyl) phosphate or TCPP is one of the most abundant organophosphate ester compounds reported in North American indoor and outdoor air, dust, and surface waters. Here we report on a series of studies conducted in Canada in which TCPP was measured in residential indoor air and dust, outdoor urban air, urban rain and streams, and final effluent from waste water treatment plants (WWTP). We have used outdoor urban air concentrations to estimate total TCPP emissions to outdoor air and the fate of TCPP in Toronto using the Multimedia Urban Model (MUM) of Diamond and co-workers. TCPP concentrations in indoor air comprised >60% of Σ_6OPEs measured, which were nearly 400 times higher than Σ_{15}PBDE concentrations measured in the same homes. TCPP concentrations in three samples of spray and rigid foam were 2.6% (7-year old rigid foam), 12% (new spray foam) and 26% (new spray foam). TCPP in PUF insulation was the putative source of TCPP in a highly insulated home. Concentrations of TCPP reached μg/L levels at low and high flow in Toronto streams which is consistent with runoff from urban sources. Concentrations were not statistically different at low and high flows nor between the three streams sampled indicating the diffuse nature of inputs. Rain concentrations contributed significantly to stream inputs. Median and maximum concentrations of TCPP in final WWTP effluent were similar to those of streams. Using MUM, we estimated total aggregate emissions of 180 g/h or ~1600 kg/y to Toronto air. 70% of TCPP was estimated to be advected downwind whereas total losses due to degradation was 22% of inputs. Due to TCPP's high solubility, 8% of total emissions was estimated to enter surface waters (streams) via accumulation and wash-off of surface films on impervious surfaces, followed by wash-off from soils. This input resulted in water concentrations comparable to those reported here. TCPP is ubiquitous indoors and outdoors. The relatively high vapour pressure of TCPP suggests that it can be released from products into which it is added, such as building insulation, and thus give rise to its abundance in indoor air. In turn, indoor losses to outdoors, as well as other potential emissions, support its abundance in outdoor air. Modelling suggests that transfer from urban air is sufficient to supply urban streams with μg/L concentrations that could be of concern to aquatic biota.

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Emerging Atmospheric Contaminants in the Canadian Great Lakes Basin

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Atmospheric pollutants were measured in the Canadian Great Lakes Basin (GLB) to assess their transport and deposition to the lakes. Started in 2005 and 2008, respectively, polybrominated diphenyl ethers (PBDEs) and other halogenated flame retardants (HFRs) were monitored in samples collected using high volume air samplers (hivols) at 3 land-based stations. Four additional land-based stations using XAD-based passive air samplers (PASs) were initiated in 2014. Neutral per- and polyfluoroalkyl substances (PFASs), organophosphate esters (OPEs) and HFRs were determined in samples collected at these PAS sites to assess their spatial distribution. Ship-based samples collected under the Cooperative Science and Monitoring Initiative (CSMI) with hivols were also analyzed for OPEs. At the 3 land-based hivol stations, 15 PBDE congeners and 12 HFRs were detected. Due to high consumption of Penta-BDE in North America, BDE-47 and BDE-99 were the dominant congeners in GLB atmosphere, followed by BDE-209. For HFRs, hexabromobenzene (HBBz), pentabromotoluene (PBT), *syn-* and *anti-* dechlorane plus (*syn-* and *anti-*DDC-CO) were frequently detected. The only detectable HFRs in the XAD-PASs were ally-2,4,6-tribromophenyl ether (TBP-AE), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (TBP-DBPE) and pentabromotoluene (PBT). 6:2-, 8:2- and 10:2-fluorotelomer alcohols (FTOHs), methyl perfluorooctane sulfonamidoethanol (MeFOSE) and methyl perfluorooctane sulfonamide (MeFOSA) were detectable at all 4 PAS sites. Also, 4 OPEs were detected in the XAD-PAS samples at levels much higher than the HFRs. Although it was generally believe that OPEs are mostly particle-bound, their detection in XAD-PAS implies that some of these compounds occur in the gas phase. The triphenyl phosphate (TPhP) was the most detected OPE in the ship-based samples but levels were quite variable. Urban centres, even smaller urban centres, have an influence on the local air concentrations of OPEs. Samples taken on Lake Ontario had higher air concentrations in the densely-populated western end of the lake compared to the central and eastern areas. On Lake Superior, samples taken close to Sault St Marie and Thunder Bay were higher compared to open lake samples. OPEs in Great Lakes air were very high compared to other airborne contaminants studied here, understanding the gas-particle distribution of these compounds will help us understand the transport and ultimately their fate in the Great Lakes Basin.

Human health: risk assessment of chemicals and PM2.5 in the environment: approaches and case studies

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Exposure to heavy metals, contaminated soil, diet and children neurodevelopment
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The objective of this work is to unravel potential associations between exposure to metals through the diet and modifiers thereof on neurodevelopmental cognitive functions of children living close a waste management plant in Greece. To this end we made use of the HERACLES-Waste study, a Greek cohort aiming at assessing the contribution of heavy metals contaminated soil, diet and children neurodevelopment. The HERACLES-Waste study has been established in 2012. Around 350 children aged 3 to 8 living close to Athens (Greece) in the proximity of the waste management plant (between 0.5 to 12 km) were enrolled. Human biomonitoring samples were collected and analyzed for metals content (Cd, Hg and As in urine, Pb in blood, Mn and Hg in hair). In addition, heavy metals soil contamination was investigated. Socio-demographic parameters such as socioeconomic status, mother and father education and stress event intensity, child anthropometric parameters and post-delivery factors including child body mass index, gender and breastfeeding were collected and analysed. Frequency food questionnaires were also filled in by the children parents in order to draw the picture of the dietary habits. The health outcomes considered are relevant to the neurodevelopmental scores in children. An Environment Wide Association Study (EWAS) approach has been followed to unravel potential associations between exposure and health outcomes. Among the investigated parameters, specific dietary components seem to have either a positive or a negative influence. Among the human biomonitoring data blood concentration of lead and of selenium appears to influence many tests outcomes showing, however opposite effects: higher blood concentration of lead are associated with worst performances in the tests administered while selenium in blood appears to produce positive effects. This result is confirmed by a number of research studies which indicate exposure to lead as one of the most environmental determinants of neurodevelopmental disorders in children. In addition, distance from the waste management site has positive effects in all kind of neurodevelopmental scores, while the opposite occurs for heavy metals in the soil. Among the exposure modifiers socio-economic-cultural factors and area of residence show important associations with the cognitive functions of the children.

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Indoor Sources of and Human Exposure to Brominated Flame Retardants (BFRs) and Phthalate Esters (PAEs)
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Indoor concentrations of, and exposures to brominated flame retardants (BFRs) and phthalate plasticizers (PAEs) are influenced by poorly understood sources and, in some cases, exposure pathways. Furthermore, their physical-chemical properties vary greatly, influencing their indoor partitioning and residence time, and hence indoor levels, persistence and exposure. Here we report on a study of 51 participants residing in the Greater Toronto Area and Ottawa, Canada. Our goal was to characterize indoor concentrations and to estimate exposure of participants to BFRs and PAEs. In the bedroom and most used room we collected and analysed floor dust and air concentrations using two types of passive air samplers (Harner-type polyurethane foam, PUF, and polydimethylsiloxane, PDMS). We also wiped the surfaces of electronic products to assess their potential for contributing to indoor BFR and PAE concentrations, as well as hand wipes of participants to estimate potential dermal and hand-to-mouth exposure. A questionnaire was developed and administered to gather personal demographic, lifestyle data, and environmental/household characteristics. PAEs were found at concentrations ~2, 3, and 4 orders of magnitude higher than BFRs in household dust, hand wipes, and air, respectively. Analysis of PUF and PDMS samples showed that TBB was the most abundant novel-BFRs (NBFRs) in indoor air followed by ATE, then PBBz and PBT. BDE-47 was the major PBDE congener followed by BDE-99 > BDE-28. PAE abundance was DEP, DiBP > DnBP > DEHP > DiNP. As expected, dust samples showed different patterns than air. NBFRs concentrations fell in the order of TBPH > TBB > DBDPE. Following the significant dominance of BDE-209 were BDE-99 > BDE-47 > BDE-100, -153. PAEs concentrations in dust were in the order of DiNP > DEHP > DnBP > BzBP. Hand wipes showed similar profiles of PAEs and NBFRs as in dust samples. BDE-209 was also the dominant PBDE congener, followed by BDE-47, -99 > BDE-49, -66, 85, -154. These data were used to estimate participants' external exposures via inhalation, dust ingestion and hand-to-mouth transfer. Exposure to PAEs via hand-to-mouth contact exceeded that via dust ingestion by 1.5 times, while the contribution of dust ingestion to BFRs exposure was about 2 times of that via hand-to-mouth contact. Both dust ingestion and hand-to-mouth exposures exceeded exposure via inhalation of all compounds by ~10 and 100 times for PAEs and BFRs, respectively.

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SAFR®: Applying a new hazard and exposure assessment approach for responsible fire safety solutions
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The increased use of plastics, foams and synthetic fibre-based fillings has transformed our spaces into practical and comfortable living, but it has also brought an increased risk of fire as many of these materials are combustible. Flame retardants enable inherently flammable materials to meet rigorous fire safety standards. From everyday electronics to airplane plastics and cinema seating, flame retarded materials are an essential part of safe modern living. Nevertheless, fire safety should not compromise safety for human health and the environment.\n\nAs part of its commitment to sustainability, ICL-IP has developed an assessment tool, SAFR® (Systematic Assessment for Flame Retardants) for a holistic evaluation of hazard and exposure of flame retardant products. Although the framework was developed for flame retardants, the methodology may be applied to a range of chemicals that are used in different final products with possible need to adapt the exposure component according to the relevant application.\n\nThe SAFR® methodology improves upon existing hazard-based approaches by incorporating an estimated exposure component based on the level of contact and measurable potential releases of flame retardants during their use. The combination of hazard and exposure provides a more complete assessment of how potential hazards translate into actual risk to humans or the environment during the intended use of the final product (e.g. TV, computer, upholstered furniture, building and construction material). As such, SAFR® addresses market needs where manufacturers of goods have to make decisions at early stages of their design phase. \n\n

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Sex-specific Associations between Particulate Matter Exposure and Gene Expression in Independent Discovery and Validation Cohorts of Middle-aged Men and Women
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Background Particulate matter (PM) exposure leads to premature death worldwide, mainly due to effects on the respiratory and cardiovascular systems. Genome-wide gene expression changes that reveal the long-term effects of particulate exposure are unknown. **Objectives** Identification of transcriptomic biomarkers of air pollution exposure and effect in a healthy adult population. **Methods** Microarray analyses were performed in 98 healthy volunteers (48 men, 50 women). 8 sex-specific candidate biomarker genes, significantly deregulated in association with long-term PM_{10} exposure in the discovery cohort and with a reported link to air pollution-related disease were measured with qPCR in an independent validation cohort (n=169; 75 men, 94 women). Pathway analysis was performed using Gene Set Enrichment Analysis. Daily $PM_{2.5}$ and PM_{10} exposure levels over a 2-year period were obtained for each participant's residential address using a spatiotemporal interpolation method in combination with a dispersion model to estimate long-term residential exposure. **Results** Long-term exposure to PM_{10} averaged (SD) 25.9 (5.4) $\mu g/m^3$ in the discovery cohort and 23.7 (2.3) $\mu g/m^3$ in the validation cohort. In discovery analysis, associations between PM_{10} and the expression of individual genes differed by sex. In the validation cohort, long-term PM_{10} exposure was associated with significant differences in the expression of the candidate genes *DNAJB5* and *EAPP* in men and *ARHGAP4* borderline significant (p=0.053) in women. Candidate genes *AKAP6* and *LIMK1* were significantly associated with long-term PM_{10} exposure although regulation differed in direction between the discovery and validation cohorts. Expression of the 8 candidate genes identified for each sex in the discovery cohort successfully differentiated between validation cohort participants with high (>75 percentile of exposure) *vs* low (< 75 pct) long-term PM_{10} exposure, with an area under the receiver operating curve of 0.92 (95% CI: 0.85, 1.00; p=0.0002) in men and 0.86 (95% CI: 0.76, 0.96; p=0.004) in women, respectively. **Conclusions** Expression of the sex-specific candidate genes identified in the discovery population predicted PM_{10} exposure in an independent cohort of adults from the same area. Confirmation of these findings in other populations may further support this as a new approach for exposure assessment, and may contribute to the discovery of molecular mechanisms leading to PM-induced health effects.

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Spatial variation of secondary inorganic PM2.5 exposure: from exposure magnitude to exposure distance
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Intake fraction (iF), the fraction of emissions inhaled by the exposed population from a given source, links environmental emissions to human exposure and is influenced by the size of exposued population and the source characteristics. Estimates for secondary fine particulate matter ($PM_{2.5}$) iFs - spatial variability in travel distances and $PM_{2.5}$ exposure due to precursors such as NH_3, NOx, and SO_2 - are not well defined. We estimate spatially-explicit $iF_{PM2.5}$ for NH_3, NOx, and SO_2, emissions in U.S. and investigate how far from the source emissions can affect population. We use a source-receptor (S-R) matrix based on the Intervention Model for Air Pollution (InMAP), an air quality model with reduced complexity. We analyze 40,000 source locations in the U.S. and estimate $PM_{2.5}$ iF from ground level NH_3, NOx, and SO_2 emissions. We also determine how far from the source we achieve x% of total $PM_{2.5}$ mass change as a result of a precursor emission, mass travel distance of x (MTDx),and x% of total $PM_{2.5}$ iF as a result of precursor emissions, intake travel distance of x (ITDx). Estimates of $PM_{2.5}$ intake induced by NH_3 emissionsindicate substantial spatial variability with values ranging between 0.03 and 14.0 ppm. Sources in CA, TX, GA, FL, and in the East North Central and Middle Atlantic regions result in the highest $iF_{PM2.5,NH3,}$ reflecting population distribution. Same trends were observed for NOx and SO_2 emissions (0.003-2.2 ppm and 0.02-4.0 ppm, respectively). In regards to exposure potential as a function of distance, MTD50 from NH_3 ranges between 65 and 950 km and has low correlation with the $iF_{PM2.5,NH3}$. The corresponding $MTD50_{PM2.5}$ estimates for NOx and SO_2 emissions were estimated at 90-1460 and 105-1460 km, respectively. Finally, the mean value of $ITD50_{PM2.5,NH3}$ is estimated at 90 km (GSD^2=4.4) while this estimate is substantially larger for SO_2 (190 km, GSD^2=4.1) and lower for NO_x emissions (15 km, GSD^2=13.5). Our results support precursor-specific spatial variations of secondary inorganic $PM_{2.5}$ exposure in the U.S. linked to population density. This work adds to previous work on $iFPM_{2.5}$ due to precursor emissions, especially for NH_3 for which earlier estimates were inadequate. . In addition, the study of mass and intake travel distances provide additional understanding of secondary PM2.5 exposure showing important variations depending on both precursor and emission location.

Improving the environmental assessment of complex composition substances and mixtures for Chemicals Management

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An improved approach for the ecological risk assessment of UVCBs at Environment and Climate Change Canada (ECCC)
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Ecological risk assessment of complex multi-component substances (e.g. UVCBs - Unknown or Variable Composition, Complex Reaction Products or Biological Materials) pose a challenge to regulators due to these substances' variable and often unknown composition, including both identity and quantity of chemical species present in the UVCB substance being evaluated. There are additional challenges in consistently assessing these substances considering the differing levels of complexity and availability and quality of data associated with each UVCB. As part of the next phase of the Chemicals Management Plan (CMP), Environment and Climate Change Canada (ECCC) is continuing to develop and refine a reliable and consistent method for the ecological risk assessment of UVCBs under the CMP. In principle, the approach taken for assessing UVCBs is the same as for discrete chemicals where we consider multiple lines of evidence in a weight of evidence approach when assessing a substance's potential to cause harm under the Canadian Environmental Protection Act, 1999. However, in practice, to evaluate the fate, exposure and effects of a UVCB, data for the UVCB substance as a whole must be evaluated (e.g., whole-substance toxicity tests), data available on individual components, along with chemical characterization information as a single line of evidence. Thus, there is often a requirement to understand how significant a contribution each major component may have in the UVCB to the endpoint or property being evaluated. In order to guide the overall assessment approach, given that the complexity of the UVCB and both the amount and quality of data available will vary, the WHO/IPCS framework for the risk assessment of combined exposure to multiple chemicals, which uses a tiered approach to both exposure and hazard assessment, is considered. The framework was designed to be additionally developed through pragmatic application in case studies. We plan to present an improved generic approach for risk assessment of UVCBs for feedback and discussion from the scientists and regulators present at this session.

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Risk assessment and management of UVCBs with the REACH Regulation
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The REACH Regulation establishes procedures for collecting and assessing information on the properties and hazards of substances. The burden of proof to identify and manage the risks linked to use of substances is placed on industry. **Figure 1: Overall process related to information requirements and Chemical Safety Assessment under REACH As for any other substances falling within the scope of REACH Regulation, substances of <strong>U**nknown or **V**ariable composition, **C**omplex reaction products or **B**iological materials (UVCB) need to be registered, with a Chemical Safety Assessment when necessary. There is a degree of established practice on how to address these substances under the REACH Regulation. As the UVCB substance might consist of constituents with different properties leading to different fate/distribution in environment, it is important to ensure that the composition of the sample (e.g. individual structure or fraction of similar structures) used for fate and exposure assessment is comparable to the composition used for hazard assessment. This enables the comparison of the Predicted Environmental Concentrations with Predicted No Effect Concentrations for the risk characterisation to be meaningful. Similarly for PBT/vPvB assessment, another step of the Chemical Safety Assessment, the compositions used for P, for B and for T assessments should be comparable. For PBT/vPvB and risk assessment various approaches can be used: ***"Known constituents" "Fraction (or block) profiling"*** *(particularly applicable to complex UVCBs)* ***Whole substance approach Combination of approaches*** Certain types of UVCB substances are addressed as groups with co-operation between ECHA, member states regulatory bodies and industry. ECHA also provides support by developing advisory documents and tools to facilitate intelligent use of information in the registration dossier and to improve the quality and relevance of the information provided by registrants. IUCLID 6 and Chesar 3 tools have been extended with the *assessment entity* concept to support transparent reporting of substance properties in a format most useful for Chemical Safety Assessment. There is considerable knowledge and experience on how to address UVCB substances under REACH/CLP. However, there is no unique tailored approach to risk assessment of UVCBs, because different approaches will be necessary for the various types of UVCB substance.

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Report on the RIFM/ECETOC Workshop: Developing a strategy to improve the environmental risk assessment of difficult to test multi-component substances
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Assessment Division; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; K. Jenner, Givaudan; E. Salinas, BASF SE / Experimental Ecotoxicology
An international workshop was held on 2 – 4 November 2016 prior to the SETAC World Congress in Orlando, FL to address risk assessment challenges for complex mixtures of substances (e.g., multi-constituent substances (MCSs), substances of unknown or variable composition, complex reaction products or biological materials (UVCBs)). International regulatory frameworks (specifically REACH, Canada's DSL Categorization and Chemicals Management Plan assessments, and USEPA's Premanufacturing Notification (PMN) process) have highlighted the complexities of registering, characterizing fate, exposure, hazard and ultimately assessing the risk of complex chemical mixtures. Several industrial sectors have developed frameworks and methodologies for characterization and analysis of these complex substances. This workshop was designed to identify best practices and key research needs to support environmental risk assessment. Multi-constituent and UVCB substances are comprised of individual constituents which may possess different physico-chemical and fate properties, meaning that the environmental risk assessment should ideally be based on a constituent approach, i.e., either based on groups of constituents with similar environmental fate and toxicity properties or on individual constituents that contribute most to the potential hazard and risk of the mixture. This approach is challenging for substances that are not well-characterised and/or have a significant fraction of "unknown" constituents. The workshop participants agreed that there is a need for a succinct, peer-reviewed publication that will highlight the current status and needs related to risk assessment for difficult-to-test multi component substances. This will include some basic discussions of the definitions and scope of substances classified as UVCBs and MCSs. In addition, emphasis will be given to evaluation of tiered strategies / approaches as well as characterization of uncertainty and variability within the various methodologies. Follow-up work, potentially comprised of peer-reviewed manuscripts, will focus on highlighting key principles from selected case examples, where various approaches were employed and evaluated for specific, real-world UVCB and MCS examples. This distillation of principles will help identify challenges and needs, and highlight specific research needs, including method development, effects-driven approaches, models, and other tools.

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Unknown or Variable Composition, Complex reaction products or Biological materials and still the same; is this possible?
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The chemical composition of UVCB substances (Unknown, of Variable Composition, Complex reaction products or Biological materials) by definition cannot be fully characterised and cannot be represented by unique chemical structures. UVCBs pose a challenge to regulation; for example, REACH chemical safety assessment depends on an understanding of composition of the substance, and the properties of constituents or blocks of constituents. These affect both the hazard profile and exposure (and therefore any risk) of the constituents. The consideration of the variability of composition of UVCBs has not been adequately addressed in guidance. Despite that samples are currently supposed to be representative of the same CAS/EC number substance; this is in practice not easy to verify. Due to developments in analytical chemistry such as LC-MS and GC-GC, the composition of many UVCBs can be established to a degree that could not have been envisaged when techniques such as hydrocarbon blocking were put forward. Therefore, it is now the case that blocks tend to be based on constituents present and representative structures, rather than physicochemical characterisation, which was once the case. How can quantification of similarity of UVCBs be achieved? The developments in analytical characterisation now enable the consideration of this question. Are materials similar enough to be called 'the same'? Statistical methods are needed to address this. The methods need to work for two up to very many sample sets. In this work, we report several novel approaches to the statistical analysis of the sameness of UVCB or multi-constituent substances, and how the outputs of these methods compare to the expert judgement of analysts. The approaches presented utilise a variety of methods developed from the principles of analysis of variance and the 'weighted Euclidean distance'. When used, these techniques need to be investigated and then calibrated. When analyses of many samples of a substance are available, methods such as the calculation of k-mean grouping can allow the assessment of whether there are one, several or many groups within the samples, and at what level of statistical significance differences are found. Petroleum substances are known UVCBs and as such, investigation of their properties from an analytical and statistical perspective is an important illustration of the new principles that are set out.

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Addressing combined toxicity in the environmental risk assessment of inorganic UVCBs
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There is a need to include a generic approach to address combined toxicity when assessing multi-metallic substances such as inorganic UVCBs (iUVCBs). Because the variability in composition is generally too large, it is not possible to identify a set of samples that would be representative and conservative for the hazard identification of the iUVCB and hence experimental testing is difficult. For this reason, the iUVCBs risk assessment is conducted based on parallel hazard, exposure and risk assessments of the constituents. Upon ECHA's request, registrants of iUVCB intermediates committed to improve the the combined toxicity assessment part of their REACH registration dossiers. An appropriate standard approach to address the combined toxicity of inorganic constituents in a regulatory framework is, however, still missing. Most standard approaches yield indeed over-conservative results such as risk scenarios at natural background concentrations when several metals are combined. Several issues that have been adressed in individual metal assessments, reappear when results are combined, e.g. dealing with natural background concentrations, essentiality, bioavailability. This makes conclusions on a generic combined risk assessment approach, i.e. extrapolated to any combination of metals and to all organisms within an environmental compartment, challenging. A generic tiered approach has been proposed for the environmental risk assessment of iUVCBs. This approach starts from the standard concentration addition evaluation based on summation of the PEC/PNEC ratios of the individual constituents and includes several options for refinement of this standard approach related to either hazard assessment (PNEC), exposure assessment (PEC) or the approach for calculating combined toxicity. Antagonism due to competition reactions between metals should also be considered for combined risk assessment because experimental data show that an additive approach is generally over-conservative for metals. In order to address this, a review of interactions observed for chronic ecotoxicity studies with metal mixtures is being prepared and the potential of modeling metal interactions will be further assessed. This presentation will present the outcomes and status of ongoing work and learning-lessons of the application of this tiered approach for addressing the combined toxicity in a constituents-based environmental risk assessment for some typical iUVCBs.

Microplastics, nanoplastics and co-contaminants: Fate, effects and risk assessment for biota, the environment and human health (III)

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Do microplastics contaminate the atmospheric environment?
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Microplastics - microscopic pieces of degraded plastic litter, or purposefully manufactured microbeads - are a pollutant of increasing environmental concern. Recently, plastic microfibers were measured in total atmospheric deposition in Paris, suggesting they occur in the atmospheric environment. If inhaled, microplastics may exert localised particle toxicity by inducing or enhancing an existing immune response. Chemical toxicity could arise due to the localised leaching of additives or pollutants adsorbed from the environment. However, our knowledge of exposure levels is poorly understood. The current study therefore aims to investigate whether microplastics are airborne, and assess the level of population exposure via two objectives: 1) adapt the Partisol air sampler for use in capturing and observing potential ambient microplastics and 2) develop Raman microscopy as a technique to detect microplastics directly on air filters. Raman microscopy is being optimised in-house to observe and identify potential microplastics directly on air filters. Currently, we have determined an optimum sampling substrate and size limit of detection against this background. Positive controls, consisting of ambient particulate matter spiked with reference plastic microbeads, will be analysed to assess detectability against background particles. Pilot environmental samples with then be taken. Collected filters will be visually analysed by imaging, sizing and classifying suspicious particles using Raman microscopy. Additionally, random areas will be scanned in the StreamLine™ scanning mode to determine whether observer bias is leading to an underestimation, as inconspicuous microplastics may be overlooked when amongst ambient particulate matter. The results will consolidate a robust method for sampling and analysing airborne microplastics, which can be adopted globally where air quality monitoring is performed. Results will also help inform policy, exemplified by the recent UK Government microbead ban, and indicate human exposure levels leading into future toxicology studies.

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What happens to polyethylene in the ocean?
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Plastic pollution is ubiquitous in the ocean and plastic types with a density lighter than water have been shown to follow the currents far away from their original source. The ocean is however a dynamic system and the particles that enter are subjected to biofouling, biotransformation and degradation. All of these processes will affect the plastic, both in terms of fragmentation, transportation and fate and plastic, also types with lighter densities such as polyethylene which is commonly

found in surface samples, could then sink to the seafloor. Polyethylene was in fact found at the bottom of the sea already in 1975. A deeper understanding of these processes is imperative in order to design relevant monitoring programs and to interpret data from field-studies. A mesocosm experiment was therefore set up in June 2016 in the Gullmar fjord, Sweden. Polyethylene film without additives was pre-degraded to four different levels and added to stainless steel cages hanging in the water. Samples were taken every four weeks and analyzed to assess biofouling, degradation, fragmentation and changes in density. Already after four weeks the samples showed a 21% coverage of biofilm, which had increased to 38% after 8 weeks. The density increased with degradation and continued to increase throughout the tests, also when the biofilm was removed. Tests with FTIR showed changes among the carbonyl groups in the material that had been pre-degraded after four weeks in the water, it also revealed changes in crystallinity. The results indicate that degradation and biofouling will rapidly affect the floating capacity of the plastic in coastal areas and highlight the importance of including these factors to better interpret field collected samples and to predict the transportation and ultimate fate of plastic material in the ocean.

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Marine snows: a vector of transport of microplastic to the benthos and its biological implications.

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Marine microplastics are so abundant, widespread and impacting on our marine environment that they have recently been suggested to represent a planetary boundary threat, alongside climate change and ocean acidification, yet our understanding of microplastic distributions and impacts remains limited. Whilst most of the research focus to date has look at the surface waters studies have shown benthic plastics can outweigh surface plastics by 400 times and indeed both deep sea sediments and organisms are now being found with microplastics in, including buoyant plastic types. We have been investigating marine snows as a potential vector for transporting microplastics of differing densities to the benthos. A range of plastics with varying forms (fragment, bead and fibre), polymers (PVC, PP, PA, PE, PS), and sizes (6 - 3000µm) were incorporated into marine snows using natural seawater placed in a tabletop roller. We demonstrate that both positively buoyant and negatively buoyant microplastic particles can be incorporated into marine snow; altering sinking rates and therefore their partitioning in the water column and importantly causing floating plastics to sink. We then tested whether plastics are more readily available to benthic filter feeders when incorporated into marine snow using a novel 2m feeding chamber to simulate a subtidal environment. We also show the incorporation of microplastics into marine snows alters the amount of plastics taken up by the filtering feeding mussel *Mytilus edulis* both through the change in sinking rate and in the bioconcentration of plastics within marine snows. Mussels were shown to uptake more plastic when incorporated into marine snows than when placed with just free plastics. Our work provides novel evidence that marine snows are a viable vector for the transport of microplastic pollution from the surface waters to the benthos including plastics from the buoyant fraction and this mechanism will change the partitioning and uptake of microplastic pollution in the water column.

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Desorption of organic wastewater contaminants from microplastic particles in presence of natural dissolved substances

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Microplastic particles (MP) are ubiquitously detected within all environmental compartments. Besides likely impairments due to their mere presence they might impact the ecosystem through leaching of additives and facilitated transport of contaminants. Several studies showing their potential to take up hydrophobic organic contaminants have been published. However, sorption behaviour of contaminants to MP in presence of further natural material, e.g. to reflect environmental conditions in freshwaters, has been neglected so far. Therefore, this study aims to investigate the sorption properties of MP and their changes due to the presence of natural occurring sorbents. As representative plastic material, polyethylene which is among the most produced and detected plastic types was chosen. Humic acid (HA) is a common type of dissolved organic matter and was hence used as a typical natural material. Sorption interactions were studied in batch experiments with a constant concentration of MP (1 g/L) and different concentrations of HA (0, 0.15, 0.25, 0.50, 0.75, 1.00 g/L). MP were previously spiked with common wastewater pollutants allowing both to study desorption from MP and to monitor sorption to HA in dependence of its concentrations. Samples were taken at 12 times from 10 min to 240 h. Log partition coefficients between MP and water were 3.98, 4.46, and 3.79 for phenanthrene, tonalide, and 4-*n*-nonylphenol (4-*n*-NP), respectively. The mean log partition coefficients between HA and water were, 3.74 for phenanthrene, 3.92 for tonalide, and 4.02 for 4-*n*-NP. In equilibrium, increasing amounts of HA go along with a decreasing fraction sorbed to MP and a decreasing freely dissolved fraction, but an increasing total dissolved fraction. Considering this, the partitioning between MP and water is remarkably influenced by the present amount of HA. Further analysis of the kinetics revealed a faster desorption from MP but also an extended time to reach equilibrium due to enlarged mass transfer. Additional modelling indicated that the external mass transfer resistance, i.e. the thickness of the water boundary layer around the MP, limited the diffusion if no or small amounts of HA are present. With increasing amounts of HA, desorption kinetics was more and more governed by intrapolymer diffusion, i.e. the diffusive flux within the MP is decisive. Thus, high amounts of HA in freshwaters lead to an increased release of pollutants and, however cause a shift towards slower kinetics.

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Do microplastics really act as vectors of organic pollutants to marine zooplankton? Experiments with pyrene and nonylphenol spiked polyethylene using Paracentrotus lividus larvae.

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Previous work has demonstrated the ingestion of microplastics (MPs) by marine zooplankton and raised concern about the potential role of MPs as vectors of adsorbed non-polar organic toxicants to planktonic filter feeders. We have tested this hypothesis using a standard marine toxicity test, the sea-urchin embryo test, modified to introduce into the incubation vials and keep in suspension low-density polyethylene microparticles of 2 to 20 μm, the appropriate size range to be ingested by these organisms. Apart from sea-water and solvent controls, and waterborne toxicants, additional treatments include virgin MPs and MPs previously spiked with several well known non-polar organic toxicants, including pyrene and 4-nonylphenol. The experimental set-up included dosage of the toxicants at two levels (ca 1/10 x and 1x toxicity threshold for this test species) via both dissolved phase and in the presence of MPs. Microscopic observation documented ingestion by the presence of larval stomachs filled with MPs. However, in none of the experiments up to date the presence of MPs increased the toxicity of the non-polar pollutants to these highly sensitive biological models, compared to the waterborne treatments. These results do not support any role for MPs as vectors of potentially harmful organic pollutants to zooplanktonic organisms.

Increasing the relevance of toxicity assessment in LCA: in the need for a cross fertilization between RA and LCA (II)

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Harmonizing human exposure and toxicity characterization

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The UNEP-SETAC Life Cycle Initiative has launched a project to provide global guidance and build consensus on environmental life cycle impact assessment (LCIA) indicators. Human health effects from exposure to toxic chemicals was selected as impact category due to high relevance of human toxicity impacts, past and present efforts in human toxicity assessment, and the need for further harmonization and global guidance. To address this need, an expert workshop was implemented in Utrecht in October 2016 with the aim of building a roadmap for a reliable and consistent approach for improving and harmonizing human toxicity characterization in LCIA. Building on initial work for the far-field and indoor air environments, and combining it with latest work on near-field consumer and occupational exposure assessment, dose-response and severity data, we aim at providing revised guidance on the development and use of impact factors for toxic chemicals. We propose to couple fate processes in consumer and occupational environments with existing environmental compartments and processes via a consistent and mass balance-based set of transfer fractions to quantify overall aggregated exposure to toxic substances. We propose the product intake fraction (PiF) as metric linking human intake via all exposure routes to substance mass in products. Further, for fine particulate matter, a constructed integrated exposure-response model has recently been proposed and applied to calculate marginal and average health impacts, which will serve as starting point for improving toxicity dose-response. To go beyond the additivity and linearity assumptions and to address essentiality and vulnerability, we propose to account for the fraction of population that is above a certain risk threshold for the considered disease/mode of action. We finally propose to explore the possibility to expand the endpoint coverage beyond cancer and non-cancer and to differentiate between other relevant health effects. For attributing severity to mortality (and morbidity) for cancer and non-cancer diseases to damage metrics, we will need to identify severity weights for population disease incidences expressed as disability-adjusted life years (DALY). All aspects for fate and exposure outdoors, consumer and occupational exposure, toxicity effects and dose-response, and cross-cutting issues are currently being further detailed aiming at arriving at recommended factors and global guidance within the next two years.

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A method for application-specific human health risk estimation from chemical exposure in an LCA context

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Increasingly, various stakeholders require information on eco/toxicity aspects of products beyond regulatory requirements for chemicals, e.g. in the framework of Life Cycle Assessment ("LCA"). The method developed (here referred to as "ProScale") aims to be a science-based, transparent, pragmatic and generally applicable methodology for a toxicological assessment of products. It is a development of initial ideas of an approach for merging applicable information from REACH with LCA. The ProScale method has been developed in an industrial consortium with expertise both from the Life cycle assessment and Risk assessment areas. Prerequisites for the method have been to: (i) assess the relevant direct exposure potential along the whole life cycle; (ii) use existing data, e.g. REACH based; (iii) allow comparison in relation to technical performance; and (iv) be relevant for business-to-business and business-to-customer communication. The ProScale method estimates a score for a specific instance of *exposure* for each substance for a given process and exposure route expreed in an Exposure Factor (EF). The EF is modelled based on the ECETOC Targeted Risk Assessment Tier 1 approach for both worker and consumer exposure. This is combined with its corresponding *hazard* for the substance considered expressed in a ProScale Hazard Factor (HF). The HF reflects the health hazard effect severity and potency based on Hazard statement Code(s) (H-phrases) and Occupational Exposure Limits (OEL). Then it relates this score to the amount of different substances in relation to the defined unit process at hand. Subsequently, the method accounts for the amount of each unit process necessary for the fulfilment of the functional unit as defined by the product system/flow chart. This is done for all included exposure instances, so that eventually a large number of ProScale scores for all included exposure instances are making up the ProScale toxicity impact potential for the overall product system of the studied product (PSP = ProScale of Product). A product can in this context also mean a service provided by the system. The smethod structure allows for comaprisons based on the same function as for other LC indicators. Currently the basic structure is in place, and case studies are carried out and under way to demonstrate the applicability of the method.

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Learnings from LCA-based methods: should chemicals in food packaging be a priority focus to protect human health?

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Given the scale and variety of human health damage (HHD) caused by food systems, prioritization methods are urgently needed. In this study HHD is estimated for case studies on red meat and sugary sweetened beverages (SSB) packaged in high-impact polystyrene (HIPS) due to various relevant health impacts. Specifically, we aim to asses if chemicals in food packaging are important to HHD in a life cycle context. The functional unit is "daily consumption of a packaged food per person in the United States." Method developments focus on human toxicity characterization of chemicals migrating from packaging into food. Chemicals and their concentrations in HIPS were identified from regulatory lists. A new high-throughput model estimated migration into food, depending on properties of chemicals, packaging, food, and scenario, and HHD was extrapolated following LCA characterization methods. An LCA-based study on the packaged foods estimated HHD from particulate matter and chemical emissions. Finally, the HHD of consumption of red meat and SSB above the minimum risk level was estimated using novel methods by Stylianou et al. 2016 based on the Global Burden of Disease studies. Results indicate that impacts caused by consumption of food items over minimum risk are high priority for mitigating HHD, as well as associated PM2.5 emissions from agriculture. Impacts due to the chemicals migrating from HIPS into food were minor given the study's assumptions, limitations, and methods. However, calculating the HHD for migration levels at the legally allowable limits resulted in impacts three orders of magnitude greater than impacts from the assumed chemical concentrations, and thus a relevant contributor to HHD. Future work is required to quantify realistic exposure to chemicals in packaging and their potential effects in order to elucidate significance in a life cycle context. Understanding toxicity risks posed by simultaneous exposure to several chemicals at one time, all of which are below safety thresholds, requires cross-fertilization with risk and toxicity research. Lastly, the methods developed are a first step towards operationalizing LCA for practitioners to ensure that minimizing impacts on the environment and resources due to food packaging design choices do not lead to unintended health risks caused by chemicals in packaging, and vice versa that minimizing exposure to hazardous chemicals do not increase environmental damages.

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Modeling indoor occupational air emissions of nanomaterials for life-cycle assessment

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Engineered nanomaterials (ENM) provide industrial and commercial benefits across many sectors but they also raise concerns over their potential environmental and human health hazards. While the technologies that utilize ENM can be evaluated by life-cycle assessment (LCA) to determine their potential resource efficiencies, existing methodologies do not allow for the evaluation of the environmental and human health hazards posed by the emission of individual ENM. Across the life-cycle of nanotechnologies, occupational settings present scenarios where production of pristine particles with small size distributions and thus exposure may occur. Neglecting such occupational, indoor ENM emissions in a LCA may result in burden shifting from the environment to workers. Currently existing life-cycle impact assessment methods take advantage of several assumptions that conveniently describe the fate and transport of small organic molecules and metals quite well, but these methods are not appropriate for ENM. In this paper, a two-zone, *dynamic* fate and transport model is presented for use with indoor, occupational ENM airborne emissions. The fate and transport model is linked to a physiologically-based pharmacokinetic (PBPK) inhalation exposure model that considers the deposition, removal and retention of particles in the lung over time. During emission events, the fate and transport of titanium dioxide nanoparticles resulted in a distinct near-field concentration that was generally twice the concentration of the far-field. During times without ongoing emissions, the near- and far-field concentrations were equal and decreased in-step (i.e. effectively becoming a one-box model when there were no ongoing emissions). Results of a steady-state model show that near-field concentrations were underestimated by roughly 90% of the maximum concentrations calculated using the dynamic model in this abstract. Inhalation exposure and final retention of particles in the lung was significantly influenced by the magnitude of the airborne concentration. As airborne concentrations rose significantly, phagocitizing cells in the air-exchange and interstitial regions became saturated. The results of the fate and exposure model were compared with a number of emission scenarios ranging from low to medium to high emission events. A notable finding was that the retained-intake fraction was inversely related to the emission magnitudes, which is counter-intuitive to conventional thinking in LCA.

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Towards a Life Cycle Based Chemical Alternative Assessment (LCAA)

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There is a need for an operational quantitative screening-level assessment of alternatives, that is life-cycle based and able to serve both Life cycle Assessment (LCA and chemical alternatives assessment (CAA). This presentation therefore aims to develop and illustrate a new approach called "Life Cycle Based Chemical Alternative Assessment (LCAA)" that will quantify exposure and life cycle impacts consistently and efficiently over the main life cycle stages. The new LCAA approach is illustrated though a proof-of-concept case study of alternative plasticizers in vinyl flooring. The proposed LCAA approach combines the following elements: a) The manufacturing phase chemical inventory is based on the environmental genome of industrial products database, ensuring mass and energy balance, b) near-field exposure to consumer products during the use phase is determined based on the mass of chemical ingredient in the product, first-order inter-compartmental transfer fractions and a matrix approach to determine Product Intake Fractions, and c) toxicity-related outcomes are compared with other life cycle impacts to evaluate the relevance of different impact categories for different consumer product classes. The retained case study is a comparison of two alternative plasticizers (DEHP-diethylhexyl phthalate vs. DIHP-Diisoheptyl phthalate) in vynil flooring. First order release rates of DEHP and DIHP from flooring material to indoor air are restricted, with over the first three years a maximum of 0.4% of the SVOC initial content in flooring emitted for DEHP and 1,9% for DIHP. For climate change, there is little difference between the two plasticizers, whereas compared to DEHP, DIHP impacts are reduced by a factor 10 for human health and a factor 3 for ecotoxicity. This proof of concept case study demonstrates the feasibility of combining chemical specific Life Cycle Inventory from manufacturing database with near-field exposure assessment during product use and to compare the interest of various chemical alternatives. Considering consumer exposure during use phase is essential for both LCA and ACC, the determination of Product Intake Fractions using first order transfer matrices enabling a parsimonious exposure assessment.

Environmental risk assessment of biocides: regulatory requirements, challenges and consequences

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Introductory talk: Working groups involved in the environmental assessment of biocides and latest developments of guidance and guidance-related documents
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The aim of the introductory talk is to provide an overview on the working groups (WG) of the Biocidal Products Committee (BPC) involved in environment exposure related items on biocides as well as on the development of guidance and guidance-related documents. Biocidal active substances need to be approved before an authorisation for a biocidal product containing them can be granted. Active substances are first assessed by an evaluating Member State competent authority (eCA) and the results of these evaluations are forwarded to ECHA's BPC, which prepares an opinion within 270 days. The BPC is supported by working groups, which contribute to the harmonisation of risk assessment under the Biocidal Products Regulation (BPR). The following Working Groups are involved specifically in environment related issues: BPC Environment Working Group (permanent) Ad hoc Working Group on environmental exposure (AHEE) Guidance and or guidance-related documents like the emission scenarios or emission scenario documents (ESD) are continuously being developed by different parties involved (i.e. member state, ECHA or industry). During 2016 and 2017 the focus was on the following: Revision of Volume IV Environment, Part B Risk Assessment (active substances) and preparation of the product part; Guide on product authorisation for product type 21 (antifouling products); Addendum to the OECD ESD for product type 18 (insecticides), including degradation in manure; Emission scenario document for product type 6 (preservatives for products during storage); Exposure assessment of metabolites in the terrestrial compartments; Emission scenario for the use of biocides in aquaculture in product type 3 (veterinary hygiene); Assessment of precursors of in situ generated active substances. ESD spreadsheets for member states and industry to increase efficiency, consistency and transparency.

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Comparative environmental risk assessment of biocides - a chance to get better products
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Individual environmental risk assessments, evaluating the environmental safety of biocides, are routinely performed at European level for active substances, and on member state level for products under the biocidal products regulation (BPR) EU No 528/2012. In addition, the BPR prescribes a comparative assessment to be carried out in the course of product authorisation, if a biocidal product contains one or more active substances that are Candidates for Substitution (CfS) according to Article 10 of the BPR. Potentially, the comparative assessment can lead to the decision not to authorise a product, even if the conventional risk assessment indicated acceptable risks.\nThe European Commission has published a Technical Guidance Note (TGN) on comparative assessment of biocidal products, laying out a procedure and providing definitions and concepts that are intended to guide the member states in the performance of this task. However, due to a lack of practical experience, the document is currently rather general in many points and there is a lot of room for interpretation.\nTherefore, the German environmental agency (Umweltbundesamt, UBA) commissioned a research project to analyse the existing guidance as far as the environment is concerned, to test its application to existing biocidal products and to develop contributions to an improved guidance that can be discussed between member states to promote a harmonised implementation throughout the EU.\nIn this project, the relevant content of the BPR and the TGN was analysed in order to identify areas that need further developments. Also, the existing work on comparative product risk assessment from the area of plant protection products was scrutinized. In a second step, the procedure laid out in the TGN is being tested in case studies using biocidal products registered for use in product types PT 8 (wood preservatives) and PT 18 (insecticides, acaricides) in Germany. The third step is an analysis of any deficits, missing elements or impracticalities of the currently available guidance. Finally, the project will lead to improved recommendations.\nAn analysis of the existing guidance concerning comparative assessments of biocidal products under the biocidal product regulation resulted in the identification of problematic areas. Recommendations will be developed to improve the transparency and efficiency of comparative assessment processes that aim to limit market access of products with unfavourable risk profiles.

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Managing environmental risk assessment of antifouling products - regulatory challenges
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Before the Biocide Regulation (EU) 528/2012 antifouling paints were in Denmark regulated by an executive order. Hence, environmental risk assessment was not performed for accepted active substances and antifouling products before being available at the market. Following the Biocide Regulation (EU) 528/2012 the OECD PT21 Emission Scenario Document was used for the environmental risk assessment of active substances. As the OECD scenario was not considered sufficient for authorization of antifouling products for pleasure crafts regional scenarios were developed. The intention among member states is to obtain harmonization and a simple procedure for product authorization. In order to prepare for authorization of antifouling products for pleasure crafts in Denmark there is a need for investigating how regional scenarios represent Danish marinas. Challenges within this task cover the harmonization of risk assessment for antifouling products in the Baltic Sea region and at the same time obtain acceptable protection levels for Danish marinas by use of preferably one regional scenario. A mapping of the 365 Danish marinas was conducted for the purpose of collecting information on location, dimensions, number of mooring boats and water/sediment characteristics. This information has been used to assess the level of protection for the Danish marinas obtained by the proposed regional marina scenarios. MAMPEC version 3.1 was used to calculate Predicted Environmental Concentrations (PEC) of dichlofluanid and irgarol representing a rapid degradable and a slow degradable compound in the water and sediment inside the marinas and in the surroundings for approximately half of the 365 Danish marinas. As proposed by the UK Competent Authority a cumulative probability distribution was constructed in order to compare PEC values representing Danish marinas to PEC values obtained for the Baltic and Baltic transition regional scenarios. Determining input parameters for MAMPEC representative for all Danish marinas appears to be a challenge as the marinas are divergent and not all values are available for each parameter for individual marinas. The first active substances for antifouling paints are approved under the Biocide Regulation (EU) 528/2012, and product applications are in the process of being prepared by the Industry, which demands that the authorities are in the process of obtaining knowledge and prepare for handling product applications.

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Studies to support the national environmental risk assessment on antifouling products for leisure boats
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Within the authorization of biocidal antifouling products for leisure boats (EU Biocides Regulation 528/2012) national specifics may be considered by the member states in their assessment of environmental risks on antifouling products (AFP). For Germany, nation-wide data on the inventory of leisure boats and their regional distribution are lacking so far. For that reason, a comprehensive study has been initiated funded by the Federal Environment Agency (UFOPLAN 2011, FKZ 3711 67 432) in order to quantify nationwide the amount of leisure boats at inland and coastal waters, to characterize the physical structure of marinas and to identify regional hot spots with high boat densities. Furthermore, water concentrations of antifouling active substances and selected break-down products were measured in a single-sampling survey at 50 marinas at freshwater, brackish and salt water sites to identify the active substances currently in use. Measured water concentrations of cybutryne and copper exceeded environmental quality standards at some sites, reaching highest levels in freshwater marinas. Beyond the scope of this project these data were used to calculate national consumption figures of AFP, biocidal active substances, and zinc oxide for the first time. Results were compared with those of other European countries in the context of national restrictions and bans. Assuming a moderate emission ratio of 50 % during service life, the amount of copper released from AFP reached about 15% of the yearly total copper input into German surface waters, underpinning its relevance as an emission source. Project results are published on the homepage of the Federal Environment Agency (http://www.umweltbundesamt.de/en/publikationen/sicherung-der-verlaesslichkeit-der-antifouling).

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The use of biocides to disinfect the ballast water of ships: protection of the marine environment from invasive species
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Invasive aquatic species present a major threat to the marine ecosystems, and shipping has been identified as a major pathway for introducing species to new environments. While ballast water is essential for safe and efficient shipping operations, it may pose serious ecological, economic and health problems due to the transfer of invasive species carried in the water from one region to another. These species range from bacteria, microbes and small invertebrates to eggs, cysts and larvae of various species, including fish. The transferred species may survive in the new environment to establish populations, become invasive, competing out native species and multiplying into pest proportions. To prevent the transfer and spread of invasive species, the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWC) was adopted in 2004. However, the BWC will enter into force not until 8 September 2017 as it was laid down in the BWC that “() it shall enter into force twelve months after the date on which not less than thirty States, the combined merchant fleets of which constitute not less than

thirty-five percent of the gross tonnage of the world's merchant shipping, have either signed it without reservation as to ratification, acceptance or approval, or have desposited the requisite instrument of ratification, acceptance, approval or accession()". This critical value was reached only on 8 September 2016 with the ratification of the BWC by Finland. Under the Convention, all ships are required to manage their ballast water to reach the D2-Standard to prevent the spread of invasive aquatic species. The D2-Standard is a performane standard, i.e. the discharged water shall contain less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension and less than 10 viable organisms per milliliter less than 50 micrometres in minimum dimension and greater than or equal to 10 micrometres in minimum dimension. Also several indicator microbes shall not exceed specified concentrations. To reach the standard most ships will need to install an on-board ballast water treatment system (BWMS). The BWMS have to undergo a comprehensive assessment of their potential impacts on human health and aquatic environment. The presentation will give an overview on the authorisation procedure for BWMS and the overlaps of this procedure with the authorisation process of biocides under the Biocidal Products Regulation.

Poster Abstracts

Multigenerational, epigenetic and evolutionary effects in human and environmental toxicology: from mechanisms to risk assessment (P)

MO001

Mechanisms of adaptation to metal pollution in the earthworm Lumbricus rubellus as detected by RNAseq

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The Avonmouth smelter near Bristol, UK, released large amounts of atmospheric pollution of cadmium, zinc, lead, arsenic and other metals to the surrounding area for decades before closing in 2003. In 2013 soil in the area was still heavily polluted, making it fertile ground for exploration of the effects of long term metal pollution on terrestrial species. The Avonmouth population of earthworms *L.rubellus* has successfully adapted to this heavily polluted environment. Exploring this and other extremophile populations may help understand why some species are more able to adapt to high levels of pollution than others, how much of their success depends on epigenetic gene regulation and/or genetic mutations. In this study a transplant experiment was performed using *L.rubellus* from the polluted soil near Avonmouth and a control population. The *L. rubellus* were collected and half of each group (polluted and control) was placed in control soil, the other half in polluted soil. Individuals were sampled after 3, 30 and 90 days. The phenotypes, genotypes, metal accumulation of all individuals were measured and RNAseq was performed on the RNA from 6 worms from each group from the 30 day exposure. After only 3 days of exposure to polluted soil the control earthworms had accumulated high levels of heavy metals. The polluted earthworms in polluted soil had higher amounts of heavy metals than the control *L. rubellus* placed in polluted soil. The polluted *L.rubellus* retained higher levels of heavy metals than the control earthworms even after being placed in control soil for 30 days. This implies that the adaptation strategy of the Avonmouth population does not include faster expelling of toxic metals. The gene expression of control worms placed in polluted soil became considerably more similar to polluted *L.rubellus* exposed to polluted soil, while polluted earthworms placed in control soil remained more similar to continuously pollution exposed worms. The greatest difference in gene expression was between the groups continuously exposed to control and polluted soils. These results imply that, while genetic background plays a role, the environment has a great effect on gene expression, showing the importance of epigenetic gene regulation and that the metals retained by previously exposed *L.rubellus* may continuously affect their gene expression.

MO002

A multiscale approach to decipher molecular mechanisms involved in intergenerational effects of ibuprofen on the mosquito Aedes aegypti.

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Pharmaceuticals commonly used by human populations are widely released in surface water through wastewater effluents, leading to chronic exposure of populations of aquatic organisms. The nonsteroidal anti-inflammatory ibuprofen is routinely detected in worldwide surface waters, at concentrations up to 5 μg/l, due to its massive consumption by humans. Nevertheless, chronic impact of this pharmaceutical on aquatic invertebrate populations remains poorly known. In a previous study on the model insect *Aedes aegypti*, we identified intergenerational consequences of parental chronic exposure (F0) to an environmentally relevant concentration of ibuprofen (1μg/l) on F1 progeny life-history traits. Progeny (F1) of F0 exposed individuals had an accelerated development and an increased tolerance to starvation. However, among all the different traits studied on F0 exposed individuals, only the longevity of male imagoes slightly increased. To understand the mechanistic processes underlying the direct and intergenerational impacts of ibuprofen, we combined transcriptomic, metabolomic and hormonal studies on several phases of the life cycle of F0 exposed individuals and their progeny (F1). This integrative approach revealed moderate transcriptional changes in F0 exposed larvae consistent with the pharmacological mode of action of ibuprofen. Despite the extensive study of hormonal, metabolic and transcriptional status of F0 exposed female imagoes, no perturbation of the key processes of oocytes maturation was detected. Interestingly, a crucial shift in the transcriptional and metabolic state of their F1 progeny was observed. Those alterations, which were likely driven by changes in epigenetic regulation of transcription, lead to ecdysone signalling potentiation and stress response mechanism over-representation, explaining the phenotypic modifications observed in the progeny of exposed parents. Overall, our study illustrates the importance of using a of holistic approach in mechanistic studies and emphasises the importance of considering the entire life cycle of exposed organisms, including those of their progeny, to fully understand the mode of action of pollutants. This also points out the need to develop and democratize technics allowing to deeply investigate epigenetic processes that could explain inter- and transgenerational impact of pollutants.

MO003

Integrated transcriptomics-epigenomics modulations in Graphene oxide nanomaterials' mediated transformed cells

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In the present study, we report that chronic exposure to graphene oxide nanomaterials causes malignant transformation of human lung epithelial cells (Beas2B cells). The commercially available graphene oxides [single layer graphene oxide (SLGO)] was chronically expose to Beas2B cells for 16 weeks at 0.1 mg/L. We found in soft-agar assay that SLGO caused malignant transformation of Beas2B cells. Next, we employ integrated transcriptomics-epigenomics (miRNA and lncRNA) profiling to evaluate the underlying molecular mechanisms. In summary, this study provides new evidence for carcinogenic potentiality of graphene nanomaterials and the potential role of integrated OMICS epigenetics that process.

MO004

Epigenetic changes upon multi-residue exposure to polycyclic aromatic hydrocarbons at environmental levels

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Besides genetic mechanisms, rapidly growing evidences have linked environmental pollutants (e.g. polycyclic aromatic hydrocarbons, PAH), with epigenetic variations, including changes in DNA methylation among others. To date, studies concerning the effect of PAH exposure on the global DNA methylation are limited and contradictory results have been observed. In this context, the present study was intended to study the epigenetic modification induced by multi-residue exposure to PAH, based on a rat model. Rats systemically exposed to a mixture of 16 US-EPA priority PAH for 90 days were used to assess the (hydroxy)methylation status of DNA and RNA, together with glutathione status. Our results showed an increase of GSH after chronical exposure to relatively low amounts of 16 US-EPA PAHs due to a stimulation of phase II detoxification mechanism through GSH conjugation in response to unbalance redox environment. This increased need of GSH leads to global DNA and RNA hypomethylation. In contrast, our results indicate an increased level of DNA methylation for the highest exposure levels. This can be explained by the positive association between global DNA methylation levels and B(a)P-DNA adducts. Thus, we further hypothesize that depending on the levels of exposure, PAH toxic mechanism is changing from depletion of global DNA/RNA methylation at lower levels to the formation of DNA adducts at higher levels. This hormetic response may give critical insights into the PAH epigenetic carcinogenicity.

MO005

Copper induces expression and methylation changes of homeotic genes in Crassostrea gigas embryos

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Copper contamination is widespread along coastal areas and its effects on the development of marine organisms are widely studied, especially in mollusks. Copper is known to induce developmental abnormalities in early life stages of Pacific oysters, however, the underlying molecular mechanisms are largely unknown. This study aims to better understand whether alterations in gene expression could mediate the embryotoxic effects of copper in *Crassostrea gigas,* as well as their DNA methylation level, which is known to contribute to their regulation. For that purpose, oyster embryos were exposed to 4 nominal concentrations of copper (0.1, 1, 10 and 20 µg.L^{-1} Cu^{2+}). Embryotoxicity was monitored at 24 h post fecundation (hpf) and genotoxicity at 7 hpf, respectively at D-larvae and gastrula stage. In parallel the relative expression of 16 genes coding for homeotic genes and involved in biomineralization and DNA methylation was measured at three developmental stages (3 hpf morula stage, 7 hpf gastrula stage, 24 hpf D-larvae stage) using RT-qPCR. Global DNA methylation analyses were performed by HPLC and variations in the gene-specific DNA methylation level were monitored using MeDIP-qPCR. Significant embryotoxic effect of copper appeared at 10 μg.L^{-1}, while significant genotoxic effects at a lower concentration of 1 μg.L^{-1}. All the genes presented a stage depended expression pattern, and the expression levels of four homeotic genes (*Notochord, HOXA1, HOX2, Engrailed 2*) were modified following copper exposure. Global DNA methylation (5-Methylcytidine and 5-Methyldeoxycytidine) measured by HPLC at gastrula stage didn't show significant changes between the different experimental conditions. However, exon DNA methylation and the transcripts levels were correlated for the *Notochord, HOXA1, HOX2, Engrailed 2* genes in control samples but not in copper exposed ones. Our study suggests that the embrytotoxic effects of copper in oysters could be the result of homeotic gene expression impairment through changes in DNA methylation.

MO006
Integration of Epigenomics in Systems Toxicology: Effects of BPA on DNA methylation of target promoters during early zebrafish development.
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Increasing evidence demonstrates that environmental insults occurring during the early development may have life-long effects, indicating that epigenetic mechanisms could be involved. Due to its similarities in epigenetic regulatory machinery to mammals, zebrafish is a recognized model for the analysis of epigenetic mechanisms in developmental biology and in environmental epigenetics. Therefore, we engaged in a study to evaluate the effects of Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining metabolomic, transcriptomic and epigenomic tools. Our long-term target is the fusion of multiple omic techniques to identify models to predict phenotypic traits and outcomes. The main objectives of our project are: 1) recognize target genes regulated by EDC exposures at the transcriptomic and metabolomic level; 2) develop new tools to characterize toxicity pathways and identify epigenetic biomarkers; 3) integrate metabolomic, transcriptomic and epigenetic platforms to identify whole genome molecular footprints characteristic of EDCs exposures. In the present study, we exposed zebrafish larvae to BPA from 0 to 5 dpfs, and analyzed changes in gene expression patterns by RNA-sequencing (RNA-seq) and in the metabolome by untargeted LC-MS. We have found alteration of the retinol and steroid pathways and have selected a set of regions of brain aromatase (cyp19a1b) and aldehyde dehydrogenase 1 family member A2 (aldh1a2) promoters to study DNA methylation profiles. After bisulfite treatment of DNA and subsequent amplification by PCR, amplicon sequencing was carried out to determine DNA methylation levels of these target promoters. We intend to determine the role of epigenetic mechanisms in life-long lasting effects of BPA and other endocrine disruptors. This study will increase the knowledge of regulatory mechanisms and modes of action of EDCs at a global scale, including epigenetic effects. We anticipate that studies on ecotoxicoepigenetics will assist in early detection and risk assessment of environmental emerging contaminants in the near future.
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MO007
Trans- and multigenerational impacts of micropollutants on life-history traits of the mosquito Aedes aegypti.
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Human activities lead to chronic contamination of surface waters by micropollutants. Although measured concentrations of such pollutants are often sub-lethal during acute ecotoxicity tests, consequences of populations chronic exposure to low environmental doses across multiple generations is of growing interest. Our study aimed at identifying the individual and population-level consequences of chronic water contamination by environmental concentrations of organic micropollutants on *Aedes aegypti* mosquito populations. It focused on three widespread pollutants, ibuprofen, bisphenol A and benzo[a]pyrene, commonly detected in worldwide surface waters. Life-history assays spanning the full life-cycle of exposed individuals and their progeny raised in unpolluted environment, associated with population dynamics modelling, evidenced life-history traits alterations in progenies of individuals chronically exposed to 1 μg/L ibuprofen or 0.6 μg/L benzo[a]pyrene. The progeny of individuals exposed to ibuprofen had an accelerated development while the progeny of individuals exposed to benzo[a]pyrene had a developmental acceleration associated with an increase in mortality rate during development. These life-history changes due to pollutants exposure resulted in a relatively shallow increase of *A. aegypti* asymptotic population growth rate. Multigenerational exposure for six generations revealed an evolution of population response to ibuprofen and benzo[a]pyrene across generations, leading to a loss of previously identified transgenerational effects and to the emergence of a tolerance to the bioinsecticide *Bacillus turingiensis israelensis* (Bti). This study shed light on the short and long term impacts of environmentally relevant doses of ibuprofen and benzo[a]pyrene on *A. aegypti* life-history traits and insecticide tolerance, raising unprecedented perspectives about the influence of surface water pollution on vector-control strategies. Overall, our approach highlights the importance of considering the entire life cycle of organisms, and the necessity to assess the transgenerational effects of pollutants in ecotoxicological studies for ecological risk assessment. Finally, this multigenerational study gives new insight about the influence of surface water contamination by micropollutants on microevolutionary processes.

MO008
Trans-generational effect of Piperonyl butoxide (PBO) on Daphnia magna: An extended study of OECD TG 211
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Most ecotoxicological test guidelines evaluate the effects observed only within single generation, thus disregarding those potential detrimental effects that may appear across generations. The toxicological potential of contaminants may either decrease or increase across generations or may even remain unchanged. We, in collaboration with other groups, have taken part in the development of a two generation *Daphnia magna* reproduction test using the OECD TG 211 protocol by using pyperonyl butoxide (PBO), a well-known and widely used insecticide, as test compound. To observe the real trans-generational effects, we extended the study for two further generations without PBO exposure. To this end, we performed morphology (body size, spine length etc.), body weight, reproductive potentiality, oxygen consumption, swimming behavior and molecular mechanistic endpoints (such as, global DNA methylation, selected gene expression, metabolomics tec.) analysis across the generations. In general, we found clear dose dependent alterations in almost all the measured endpoints in exposed generations (P0, F1) and recovery across generation (F2, F3) in lowest dose (25ppb). Interestingly, at the higher exposure conditions (50 and 100ppb) almost no recovery was observed, particularly in reproductive potentiality, even in unexposed generations (F2, F3) which exhibited potential trans-generation effects which remain unchanged even after withdrawing chemical exposure. In summary, our results present a paradigm not only of multigenerational exposure-effect quantification but also exhibit another important layer of observation of true trans-generational effects by combining standard toxicity endpoints with epigenetic and behavior analysis. Keyword: OECD TG 211, *Daphnia magana*, Transgenerational effect, Piperonyl butoxide

MO009
Transgenerational inheritance of reproductive defects in the crude oil exposed nematode, Caenorhabditis elegans: An epigenetic approach with various exposure scenarios
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Growing evidence indicates that environmental pollution leads to epigenetic transgenerational inheritance that influences various aspects of organismal health. To explore the long-term effects of the Hebei Spirit oil spill accident, we investigated whether exposure to a crude oil causes transgenerational toxicity in the nematode *Caenorhabditis elegans*. The effect of the crude oil on *C. elegans* growth and reproduction was investigated on the exposed generation (P0), as well as on three unexposed consecutive generations (F1-3) with three different exposure scenarios, partial early life exposure (PE), partial late life exposure (PL), and whole life exposure (WE). We found that reproduction potential was inhibited by the crude oil in the unexposed generations, specifically at F1, as well as, in the exposed parental generation. Results from different exposure scenarios indicated that PL as a sensitive window of exposure than PE, for reproduction failure, while WE exposure condition exhibited true trans-generational inheritance of defective reproduction. Epigenetic mechanism study revealed that decreased methylation of histone H3 marks (i.e., H3K9) was subsequently found in the oil-exposed generation. Further functional multi-generational investigations revealed that a heritable diminution in reproduction did not occur in the loss-of-function mutant of the gene encoding the H3K9 histone methyltransferases (HMT), met-2(n4256). This suggests a potential role for the HMT of H3K9 in transgenerational toxicity. Our overall results suggest that toxicity from crude oil could be heritable, with profound effects on reproductive function and histone methylation associated with the transmission of the inherited phenotypes. Keyword:Crude oil, *Caenorhabditis elegans* reproduction, Transgenerational effects, Differential exposure, Epigenetics, Histone methyltransferase

Advances on the assessment of environmental pollutants to amphibians and reptiles (P)

MO010
Using 1H NMR-based metabolomics to explore sub-lethal toxicity of a mixture of diabetic and lipid-regulating pharmaceuticals on amphibian larvae
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Pharmaceuticals are widely used for the treatment of various physical and

psychological ailments. The diversity of pharmaceutical contaminants and potential for complex mixtures to occur makes it very difficult to predict the toxicity of these compounds on wildlife, and robust methods are therefore needed to explore sub-lethal effects. Metabolic syndrome is one of the most widespread health concerns currently facing the human population and various drugs, including anti-diabetic medications and lipid- and cholesterol-lowering fibrates and statins, are widely prescribed as treatment. Due to incomplete removal through sewage treatment, many such contaminants have subsequently been identified in global aquatic waterways. In this study, we exposed striped marsh frog (*Limnodynastes peronii*) tadpoles to control, solvent control (methanol), and a mixture of the drugs metformin, atorvastatin and bezafibrate at 0.5, 5, 50 and 500 µg/L to explore possible effects on growth and development, energy reserves (triglycerides and cholesterol), and profiles of small polar metabolites extracted from hepatic tissues. It was hypothesised that exposure would result in a general reduction in energy reserves, and that this would subsequently correspond with reduced growth and development. Responses differed from expected outcomes based on the known mechanisms of these compounds in humans, with no changes to hepatic triglycerides or cholesterol and a general increase in mass and condition with increasing exposure concentration. Deviation from the expected response patterns may be explained by differences in the receptivity of the compounds in non-mammalian species. Interestingly, Nuclear Magnetic Resonance (NMR) spectroscopy revealed possible interactive effects between the carrier solvent and the pharmaceutical mixture. This could have important implications for studies assessing the aquatic ecotoxicity of certain pharmaceuticals, and is therefore an area that requires further investigation. Overall, the results demonstrate that it may not be possible to predict adverse toxicological effects of pharmaceuticals on non-target wildlife based on our knowledge of how these compounds act in humans.

MO011
May gold nanoparticles impair feeding and growth of Xenopus laevis tadpoles?
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Gold nanoparticles are commonly used in medical therapy and cosmetics, but the study of this nanoparticle corresponds to only 3% of the published papers focusing on engineered nanoparticles, both for freshwater and saltwater species. Thus the aim of this study was to determinate the sub lethal effects of gold nanorods (Au-NR) to tadpoles. To attain this objective, tadpoles of the amphibian species *Xenopus laevis*, at Gosner stage 25, were exposed, for 96h in the dark, to a control (FETAX medium) and to several concentrations of Au-NR. Five replicates were performed for each tested concentration and control. Four tadpoles (G25) and 6 x 10^5 cells/mL of the microalgae *Raphidocelis subcapitata* were introduced per replicate. In order to calculate the feeding rate of tadpoles, absorbance of the media was measured at the beginning and at the end of the assay. As well, tadpoles were weighted and their length measured at the beginning and at the end of the assay. The Au-NR concentration causing 50% of feeding inhibition (EC_{50}, and respective 95% confidence limits) of tadpoles was 0.004 (0.003-0.004) mg/ml. Exposure to Au-NR also caused significant effects on the length of tadpoles ($p< 0.001$). The snout vent length (SVL) of the tadpoles exposed under control (average±standard deviation: 4.64±0.07mm) conditions was significantly higher than tadpoles exposed to Au-NR (lowest tested concentration, 0.004mg/ml: 4.28±0.08 mm and highest tested concentration, 0.01mg/ml: 3,46±0.04 mm). As well, Au-NR influenced the total body length (TBL) of tadpoles ($p< 0.001$). The TBL was significantly higher for tadpoles exposed to the control (11.90±0.17 mm) comparatively to those exposed to 0.007 (11.15±0.10 mm) and 0.01(10.80±0.08 mm) mg/ml of Au-NR. Finally, obtained results showed a significant decrease in body weight of tadpoles exposed to Au-NR ($p < 0.001$): the control group weight on average 16.24±0.48 mg, while tadpoles exposed to the concentrations of Au-NR weight less than 13.2 mg. The concentrations of Au-NR causing significant effects at the monitored sublethal endpoints were quite low. However, some of this toxicity can be due to its capping agent Cetyltrimethylammonium bromide.

MO012
Effect of glyphosate and temperature on the growth of Pelophylax perezi tadpoles
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Amphibians are among the most endangered vertebrates and, besides habitat loss, some of the main factors pointed as responsible for this status are habitat contamination and climate alterations. Even though many studies have already shown that these stressors can interact and that, depending on their variation, the outcome for amphibians can be different, much work is still needed to further clarify environment and contaminants interactions. One such case, and considering the ectothermic nature of amphibians, is the effect that different temperatures will have on pesticides' toxicity. This might be quite relevant when considering widely used pesticides such as glyphosate-based pesticides. Glyphosate-based herbicides, such as Roundup®, are among the most widely used herbicide formulations. These broad-spectrum herbicides act by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) in plants, which is responsible for the synthesis of aromatic amino acids. This study aimed at assessing the influence of Roundup Ultra™, as well as its relation with temperature, on the growth of tadpoles of the Iberian green frog, *Pelophylax perezi*. To achieve this goal, *P. perezi* embryos in midblastula (stage 8) were exposed to three glyphosate concentrations: one equivalent to that found in agricultural basins (0.43mg/L), one lower (0.29mg/L) and one higher (0.65mg/L), as well as a control group (0.00mg/L), at three different temperatures (15ºC, 20ºC and 25ºC). Besides mortality, growth, and teratogenesis, the antioxidant activity of several enzymes was evaluated, as well as lipid peroxidation. The results revealed no effect of the pesticide on mortality and teratogenesis for either temperature. The results only revealed significant differences in growth between the highest concentration and the control, and only for the lowest tested temperature (15ºC), showing that animals exposed to Roundup® presented larger body sizes. These results suggest a higher metabolization and elimination of the compound at higher temperatures. The biochemical parameters will provide further comprehension of the subcellular effects resulting from the tested combined exposure. These results emphasize the importance of temperature in the effect of herbicides and that this influence should be considered in future ecotoxicological studies.

MO013
Tolerance of amphibians and its skin symbiotic bacteria to increased salinity
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Salinization of coastal freshwater ecosystems constitutes an increasing environmental concern, namely due to the predicted future increase in the mean sea level. The main goals of the present study were: (i) to assess the adverse effects caused by salinity on amphibians and its skin microbiome, (ii) to evaluate the capacity of amphibian skin bacteria to acquire increased tolerance to salinity. For this, bacterial isolates, collected from the skin of natural populations of *Pelophylax perezi*, and tadpoles of *P. perezi* and *Xenopus laevis* were exposed to serial concentrations of NaCl. Furthermore, the sensitivity of the skin bacterium isolate *Erwinia toletana* was assessed before and after being exposed, for six generations, to its EC_{10} for NaCl (18g/L). The majority of bacterial isolates seemed to be quite tolerant to NaCl, being the average value of the concentrations causing 20% of growth inhibition ($EC_{20,120h}$) of 14g/L for NaCl. Looking at the lethal sensitivity of the two species of amphibians, it was observed that both species exhibited a similar sensitivity to NaCl, but both tended to be more sensitive than the skin microbiome to increased salinity. The concentrations of NaCl causing 20% of mortality in tadpoles ($LC_{20,168h}$) were 4.61 g/L for *P. perezi* and 4.98 g/L for *X. laevis*. The bacterium *E. toletana* showed an increase in tolerance to NaCl after being exposed for six generations to the EC_{10} for NaCl, being the EC_{20} (and 95% confidence limits), before and after such exposure, of 20 (18.5-21.9) g/L and 30.8 (25.4-36.3) g/L, respectively. Furthermore, metabolic process of carbon compounds shown to be different between exposed, not exposed and recovered isolate. *Erwinia toletana* after being exposed for six generations to salinity showed differences in substrate metabolization comparing to not-exposed and recovered isolate. The NaCl exposed bacterium showed more carbon sources metabolized while recovered *E. toletana* seemed to return almost entirely to the same carbon source metabolic activity of the not-exposed isolate. It is possible that *E. toletana,* after generational exposure to increased salinity, evolved plasticity characteristics, requiring other metabolic mechanisms to tolerate salinity stress.

MO014
Interactions of environmental stressors with the amphibian pathogen Saprolegnia australis
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The risk of disease in amphibians depends on host's susceptibility, pathogen infection capacity and virulence. However, environmental perturbations and chemical contamination can induce alterations in these host-pathogen dynamics. Water mold infections are responsible for large economic losses on aquaculture. Additionally, the worldwide decline of some amphibian populations has been linked to these pathogens. They are mostly saprobes, capable of parasitism that may act as opportunistic pathogens and effects on amphibians appear to be influenced by environmental factors (*e.g.* UV-B radiation). The present work aims to understand the effects that environmental changes may cause on the amphibian pathogen *Saprolegnia australis*. To achieve this objective, ecotoxicological assays were carried out by exposing the pathogen to: i) varying NaCl concentrations (from 4.9 $mScm^{-1}$ to 54.9 $mScm^{-1}$); ii) seawater dilutions (SW), equivalent to each tested NaCl solution; iii) acid mine drainage (AMD) dilutions (from 0% to 100%) and iv) pH levels, corresponding to the different AMD pH values. Radial growth, biomass production, extracellular enzymatic activity and mycelium chemical composition

(FTIR-ATR) were assessed. Radial growth was not significantly affected when *S. australis* was exposed to the different NaCl solutions. However, a significant growth inhibition relatively to the control was observed when exposed to high AMD levels. The effective concentrations causing 20% reduction in biomass production for NaCl, SW, AMD and pH were, respectively: 47.0 $mScm^{-1}$, 41.7 $mScm^{-1}$, 19.2 %, 4.17. Both, NaCl and SW caused an increase in proteolytic activity, though lipolytic activity decreased at the highest concentration of NaCl. Tested AMD dilutions did not affect the enzymatic activity. FTIR-ATR analyses showed a decrease on the intensity of bands for the highest concentrations of NaCl, AMD and lowest values of pH. For SW, the lowest concentrations exhibited similar band pattern comparatively to the control, but intermediate concentrations caused an increase in band intensity, corresponding to an increase in proteins, lipids and polysaccharides. In conclusion, the growth of the pathogen is affected by exposure to different types of chemical contamination and, FTIR-ATR showed changes on mycelium composition. Additionally, it is important to highlight that the fact that NaCl and SW increases the proteolytic activity, it could reveal an increase of infection capacity of the oomycetes.

MO015
Current studies on ecotoxicity of accident preparedness substances in Korea
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There have been a number of chemical accidents in the forms of explosion, spill, leak, conflagration and so on in the world. In Korea, there have been several chemical accidents like leakage of hydrofluoric acid, hydrochloric acid, toluene and sulfuric acid from 2000. Korea government designated 37 accident preparedness substances (hydrogen chloride, sulfuric acid, ammonia, nitric acid, toluene, hydrogen fluoride, chlorine, methanol, methyl ethyl ketone, hydrogen peroxide, phosgene, benzyl chloride, phenol, phosphorus trichloride, arsine, chlorosulfonic acid, phosphine, phosphorus oxychloride, diborane, formaldehyde, formic acid, acrylic acid, p- nitrotoluene (solid), meta-cresol, sodium cyanide, toluene-2,4-diisocyanate, ethylenediamine, isophorone diisocyanate, benzene, methyl ethyl ketone peroxide, vinyl chloride, methylhydrazine, hydrogen cyanide, carbon disulfide, allyl chloride and propylene oxide). Among them, there are carcinogens (benzyl chloride, formaldehyde, benzene, vinyl chloride and propylene oxide). These chemicals are very toxic and dangerous materials but there are insufficient ecotoxicity data of terrestrial ecospecies up to date. We searched and listed the available ecotoxicity data of these 37 chemicals and tried to confirm the research trends of these chemicals. When the accidents occur, leaked and spilled chemicals enter the soil and affect the terrestrial ecosystems. More toxicity data are needed to predict and manage the risk of these chemicals. *Acknowledgement - This subject is supported by Korea Ministry of Environment (MOE) as "The Chemical Accident Prevention Technology Development Project."*

MO016
SETAC Interest Group on Ecotoxicology of Amphibians and Reptiles
C. Aubee, US Environmental Protection Agency / Office of Pesticide Programs

Combined effects of chemical and environmental stressors: from local stressors towards climate change (P)

MO017
Temperature-dependent effects of metal mixtures: behavioral changes in a freshwater invertebrate
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Since current environmental quality standards (EQS) are mainly based on standard toxicity tests, an accurate extrapolation from laboratory to field data is vital. However, in those standard tests, organisms are exposed to a single stressor under favorable conditions, which is not a realistic representation of the natural environment. There is increasing recognition that the combined effects of multiple stressors cannot be disregarded. Because of global warming's impact on the aquatic ecosystems, research that focuses on the combined effects of elevated temperatures and chemicals has received much attention. Yet, very few papers describe the effects of temperature on the uptake of toxicant mixtures. As animals are exposed to numerous chemicals simultaneously in nature, performing toxicity tests with both these mixtures as well as a temperature factor creates a more sensible experimental set-up. The present study investigated the sublethal effects of exposure to metal mixtures of Cd, Cu and Pb at two different temperatures (15 °C and 20 °C) on the freshwater ectotherm *Asellus aquaticus* (Isopoda). As metal concentrations, we chose the EQS, 10xEQS and 100xEQS, for Cd: 0.15, 1.5 and 15 $\mu g.L^{-1}$; for Cu: 7, 70 and 700 $\mu g.L^{-1}$; and for Pb: 7.2, 72 and 720 $\mu g.L^{-1}$. At the end of this 10-day laboratory experiment, we determined the uptake rate, respiration, growth rate and, as behavioral endpoints, activity and feeding rate. We hypothesized that an increased temperature would cause a higher respiration and thus metal uptake, which would lead to a decreased growth rate, activity and feeding rate. Behavioral changes as these could significantly affect the ecosystem by, for example, altering predator-prey interactions. By performing more realistic multiple stressor toxicity tests, chances of over- or underestimating the impact of metal exposure in nature will greatly diminish.

MO018
Effects of titanium dioxide (TiO2) nanoparticles from sunscreen on coral symbionts, Symbiodinium spp., and their combined toxicity with global warming.
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Titanium dioxide nanoparticles ($nTiO_2$) are widely used as UV-filters in sunscreen. Estimates show that between 16000 and 25000 tons of sunscreen are used in tropical countries and 25% of sunscreen ingredients are washed off from the skin during swimming. Therefore titanium nanoparticles may pose a potential major threat to marine organisms. Commonly available mineral sunscreens contain $nTiO_2$ with different surface coatings to reduce photodegradation and enhance dispersion within the organic cream. These coatings, however, may also have a role in toxicity, and by directly mediating hazard of nTiO2, or through any alterations following their interactions with the environment. The effects of three different types of titanium nanoparticles, commonly used as sunscreen ingredients, were tested using cultured coral endosymbiotic dinoflagellates (*Symbiodinium* spp.). The chosen $nTiO_2$ share the same crystal structure (rutile) but have different external coatings (dimethicone, simethicone and stearic acid). Diverse *Symbiodinium* phylotypes, known for their different tolerance to environmental change and stress, were exposed to $nTiO_2$ at both ambient temperature (26°C) and thermal stress condition (32°C) to evaluate growth rate (chlorophyll extraction as proxy for algae biomass), photosynthetic activity and reactive oxygen species (ROS) production. Results show an inhibitory effect of $nTiO_2$ on the algal growth and an increased production of ROS, proportional to the nanoparticles concentration and enhanced by high temperature but not dependent on the coating type. Coral bleaching events worldwide are increasing in intensity and severity as global temperatures continue to rise and the accumulation of ROS in *Symbiodinium* cells in response to elevated temperature stress is considered to be the trigger to coral bleaching. Thus the observed enhanced ROS production in the algal cells, derived by the simultaneous exposure to $nTiO_2$ and the sea surface temperature value projected for the end of the century, suggests that exposure to sunscreen nanoparticles may pose a risk to coral reef ecosystems by exacerbating bleaching response in corals.

MO019
Transgenerational interactions between a pesticide and warming in a vector mosquito
T. Tran, University of Leuven; L. Janssens, KULeuven; K.V. Dinh, DTU Technical University of Denmark; R. Stoks, University of Leuven / Department of Biology
Climate change imposes a strong pressure on the persistence of natural populations and together with pollution it exerts a global threat to biodiversity. While many transgenerational studies have revealed the capacity of species to adapt to a temperature increase, it remains unknown if this ability may change in a polluted environment. We set up a full-factorial transgenerational experiment where *Culex pipiens* vector mosquitoes were reared at two temperatures (20°C vs 24°C) and, when they reached the final larval stage, exposed to one of two chlorpyrifos treatments (absent vs present). We studied effects on larval survival and age and size at metamorphosis. In both generations, warming and the pesticide reduced larval survival and accelerated development in the survivors. While warming reduced size at metamorphosis, pesticide exposure did not affect size. As expected, the effect of chlorpyrifos on mortality was stronger under warming. We could show delayed effects of parental rearing temperature on their offspring with parents reared at 24°C producing offspring with a lower survival, slower development, but a larger size at metamorphosis. For survival the effect was particularly strong in offspring that was reared at 20°C, thereby providing evidence for transgenerational acclimation resulting in poor offspring performing under thermal conditions different from their parents. Parental pesticide exposure influenced the response of the offspring to both stressors, with offspring from parents exposed to the pesticide being more susceptible to warming in terms of survival, but performing better when also exposed to the pesticide in terms of size at metamorphosis. Our results indicate some signals of transgenerational acclimation to the pesticide: offspring exposed to the pesticide did better when the parents were also exposed to the pesticide. However, when combining stressors, we could show that parental pesticide exposure increased the vulnerability to warming indicating the complexity of transgenerational acclimation. This highlights the importance of looking at the combined impact of pesticides and warming increase across generations to come to a better understanding of the impact of pesticides in a warming world.

MO020
Interaction between increased temperature and Cu excess induces differential antioxidant enzymes responses in the brown macroalga Ectocarpus siliculosus
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& Engineering)
At elevated concentrations, copper (Cu) can become toxic and have detrimental effects on seaweeds and other organisms inhabiting coastal ecosystems. Brown seaweeds are major primary producers and important bio-engineers in temperate regions, providing habitat, food and shelter for a large diversity of sea life. Although some harmful effects of Cu in seaweeds, such as oxidative stress, have been described, the consequences of the interaction between Cu and other abiotic stressors remain poorly understood. Besides, the observed increase of average sea surface temperature (SST) represents an additional hazard for seaweeds. In this study, we evaluated the interactions between Cu excess and increased temperatures in some parameters related to reactive oxygen metabolism (ROM) of the model brown seaweed *Ectocarpus siliculosus*, such as antioxidant enzyme activities, content of reactive oxygen species (ROS) H_2O_2 and levels of lipid peroxidation. Algal cultures were treated for 10 days with a combination of 2 factors and 2 levels per factor: Temperature (15 and 19°C) and Cu (control and 2.4 μM). Catalase (CAT) activity was not affected under any experimental condition, whereas superoxide dismutase (SOD) activity decreased at the highest temperature (19°C), although no significant interaction with Cu was observed. For glutathione reductase (GR) and ascorbate peroxidase (APX) activities, a significant interaction was observed between stressors; the synergistic interaction between 19°C and 2.4 μM Cu was detrimental for the activity of both enzymes. These results are in agreement with H_2O_2 concentrations and the levels of lipid peroxidation (measured as thiobarbituric acid reactive substances, TBARS) after 10 days of exposure. Concentrations of both H_2O_2 and TBARS increased when stressors were applied in combination compared to the effects of individual stressors, which indicates a synergism of both factors in the stress response of *E. siliculosus*. The results also suggest that the combined detrimental effects of increased temperature and Cu excess are, at least partially, mediated by decreased APX and GR activities. Additionally, from a kinetics analysis of H_2O_2 and TBARS concentrations, we observed that the highest production of H_2O_2 and TBARS occurred between 6 and 10 days of exposure. The information suggests that after 6 days of exposure to both combined stressors, ROS levels start to overcome antioxidant defences and oxidative damage becomes a threat.

MO021
Combined effects of invasive species and insecticide exposure to freshwater benthic invertebrate community: a mesocosm approach
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Pesticide contamination and invasive species are two main anthropogenic stressors of concern in freshwaters. Thus, understanding their combined effects to community structure and vital ecosystem functions presents challenges for an improved ecological risk assessment. This study focus on community-level data obtained in artificial stream mesocosms to evaluate the direct and indirect effects of insecticide exposure and the presence of invasive species on the structure (macroinvertebrate abundance) and function (leaf degradation, primary production) of a natural benthic community, and how the presence of invasive species alters the community responses to chemical stress. For that, the neurotoxic insecticide chlorantraniliprole (CAP) was used as a model compound. Community responses to CAP-induced stress were assessed in the presence of leaf litter of different nutritional value (native *Alnus glutinosa vs* invasive *Eucalyptus globulus* leaves) and the presence of different predators (native dragonfly *Cordulegaster boltonii vs* invasive crayfish *Procambarus clarkii*). Our results showed that environmentally relevant CAP exposure caused a reduction of 4 and 10 % of shredders and collectors, respectively, with consequent reductions in leaf decomposition. These deleterious effect of CAP were magnified in the presence of Eucalyptusleaf, with shredders and collectors reduced by 8 and 30 %, respectively. CAP exposure also reduced grazers' abundance and an increase of primary production was observed in channels with Alnus leaf litter, but this indirect effect was enhanced when both Eucalyptus leaves and crayfish were present. The presence of the voracious crayfish decreased detritivore abundances, being collectors and grazers the preferential preys. Interestingly, under CAP exposure invertebrate mortality due to crayfish predation seemed to be reduced, which might be related to CAP induced effects on behaviour reducing predation risk. Structural equation modeling (SEM) highlighted the influence of leaf litter type on shredder abundance and primary production, and identified the presence of the invasive predator as the main driver of changes on collector and grazer abundances. In summary, this study reveals that detritus quality and predator identity can mediate the effects of insecticide contamination on structural and functional endpoints in benthic freshwater communities and highlights the value of incorporating biotic stressors in ecotoxicological experiments.

MO022
The common pollutant copper alters the physiological responses of the king ragworm Alitta virens to ocean acidification.
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Ocean acidification (OA) is widely regarded as one of the major threats to marine organisms, however, it is not happening alone. There are now multiple anthropogenic threats affecting the marine environment including low-level metal contamination. Metals are a very common pollutant, especially in coastal waters and sediments and OA has the potential to alter the bioavailability of pH sensitive metals, such as copper, by changing their speciation in seawater. This change in speciation under OA conditions has the potential to alter the toxicity effects in marine biota. Previous studies using mussels and sea urchins, which are sensitive to OA, have demonstrated that OA increases the toxicity of copper in a species specific way related to their OA responses. Here we test whether this paradigm holds true in a phylum less sensitive to OA, the polychaetes. The king ragworm, *Alitta virens* is a sediment dwelling polychaete found in coastal waters often contaminated with low levels of copper, making it fairly robust to pollution. We investigated whether the copper responses of *A.virens* are affected by OA by exposing them to the following treatments; pH 8.1 (ambient control), pH 8.1 + copper (0.25 μM), pH 7.7 (OA conditions) and pH 7.7 + copper. Worms' coelomic fluid pH remained relatively stable across all four treatments demonstrating that this species can acid-base regulate in the face of elevated seawater pCO2. Under OA conditions with no copper, no changes in coelomic fluid pH or bicarbonate levels were observed compared to the ambient (pH 8.1) control despite the elevated seawater pCO_2, suggesting the worms are controlling their internal pCO_2 levels by an alternative, possibly behavioural, mechanism. However, the presence of copper altered the acid base physiology of the worms, causing a decrease in both the pCO_2 and bicarbonate levels in the worms' coelomic fluid. We found a significant increase in DNA damage under copper conditions (increasing from 18.7% to 29.7% in the control pH and from 17.7% to 34 % at 7.7 pH), however, OA conditions did not increase the DNA damage induced copper to *A.virens*. This differs from the previous studies on mussels and sea urchins where OA increased amount of DNA damage induced by copper. This new evidence adds to the developing paradigm that species physiology is key in determining the interactions of these two stressors rather than it purely being driven by the changes in metal chemistry.

MO023
The combined effects of zinc and phosphorus in a predator (D. magna) - prey (P. subcapitata) microcosm
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Synergistic interactions between trace metals toxicity and phosphate deficiency have been found on growth of primary producers and were ascribed to lack of cellular phosphate to detoxify Zn^{2+}. In contrast, antagonisms of Zn stress (toxicity) and P stress (deficiency) have also been found in other studies and are explained by the Liebig concept, i.e. effects are due to one most limiting factor. How these factors interact in a multiple species ecosystem is unknown. Standard assays use adequate P supplies whereas environmental systems are commonly P-limited. This study aims at understanding the combined effect of Zn supply and P limitation on growth of primary producer *P. subcapitata* and on *D. magna* reproduction. First, dietary toxicity was studied using algae cultured at different Zn and P doses in factorial supply. Subsequently, these algae were fed to *D. magna* with no waterborne Zn and *ad libitum* algae supply. That experiment showed that the relative toxic effect of dietary Zn on *D. magna* reproduction was higher when algae cultured at a high P supply were used to feed the daphnids, compared to those cultured at low P supply. A possible explanation is the increased nutritional quality of the dietary food due to synergistic interactions between P and Zn uptake of algae cells. Second, a microcosm experiment was set up in static, non-renewed cultures using *D. magna* and *P. subcapitata.* Treatments included factorial supplies of waterborne Zn, P and initial algal cell numbers per *Daphnia* juvenile. Initial data suggest that Zn had a stronger effect on *D. magna*reproduction in the microcosm than in a classic static renewal test with regular resupplies of low Zn alga. This is likely because of the larger sensitivity of Zn to algae, indirectly affecting *Daphnia* by reduced food supply and, potentially, by the additional dietary Zn toxicity. The complete data analysis will be presented at the conference.

MO024
The effect of toxicant dispersal on the relationship between regional diversity and productivity
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The presence of chemicals in the environment generates differences in species composition among polluted and unpolluted communities. Communities are often

arranged in a network, the metacommunity, in which individuals are transferred by dispersal. Dispersal has been demonstrated to affect both among-community diversity (beta-diversity) and regional productivity. However, the relationship between both has been poorly studied. Moreover, the dispersal of toxicants is often neglected, while it can affect local environmental conditions, leading to changes in beta-diversity and productivity. Here, we examine how the dispersal of species and toxicants alters regional diversity and productivity in aquatic metacommunities. To do so, marine micro-algae and a toxicant were moved independently (following a full-factorial design) in a two-patch metacommunity. One community was unpolluted while the other contained the herbicide atrazine (250 μg/l). Algae dispersal created a gradient in beta-diversity, while toxicant dispersal homogenized the abiotic conditions in both patches. In the metacommunities with toxicant dispersal, the relationships between beta-diversity and regional productivity were positive at the start, but negative afterwards. In the metacommunities without toxicant dispersal, the relationship between beta-diversity and regional productivity was positive at the end, but insignificant before. Toxicant dispersal thus changed the relationship between beta-diversity and regional productivity. In the communities with toxicant dispersal, algae transfer from the more productive unpolluted communities to the polluted communities reduced regional productivity at a low beta-diversity at the start. Afterwards, algae dispersal increased productivity of the polluted communities in case atrazine concentrations were reduced by toxicant dispersal. Hence, regional productivity increased at a low beta-diversity. In the metacommunities with toxicant dispersal, productivity of the unpolluted community increased at the end because a strong competitor almost reached monodominance. As this effect was strongest in communities without algae dispersal, regional productivity increased at a high beta-diversity. This study shows that the dispersal of individuals and toxicants can affect toxic effects on community composition and ecosystem functions such as productivity. The incorporation of regional dynamics when examining toxicant effects on community level is thus recommended.

MO025

Contribution of natural factors and anthropogenic chemicals to the population dynamics of marine zooplankton

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Since the anthropocene, marine ecosystems have been exposed to changing environmental conditions, such as changing temperatures, sea level rise, ocean acidification, coastal eutrophication and an increasing amount of potentially hazardous chemicals. To date, the effect of multiple stressors, both environmental and human-induced, remains poorly understood and most of the knowledge available is related to phytoplankton. To partly address this knowledge gap, in the present research, we aim to assess the effect of multiple stressors on the zooplankton community in the Belgian part of the North Sea. Hence, we have quantified the relative contribution of oceanographic variables (e.g. water temperature, salinity, nutrient concentrations, pH, chlorophyll a concentrations etc.) and anthropogenic chemicals (e.g. polychlorinated biphenyls, polycyclic aromatic hydrocarbons and organic pesticides) to the zooplankton population dynamics. To do so, we applied generalized additive models to monthly zooplankton abundance data collected in 2009 and 2010 at ten sampling locations in Belgian marine waters. We found that models with temperature, salinity, turbidity, chlorophyll a concentration, nutrient information and concentrations of hazardous chemicals explained 60% to 80% of the variability in the zooplankton abundance data. Based on backward selection using the Akaike information criterion, we found that the geographical location of the sampling site (27%), chlorophyll a concentration (17%), nitrogen-phosphorus ratio (8%), water temperature (6%), salinity (5%) and concentrations of polychlorinated biphenyls (5%) were the main contributors to explain the abundances of a marine copepod, i.e. *Temora longicornis*. These findings will be validated for other zooplankton species and for other geographical regions.

MO026

Mixture of pesticides occurred in the Nakdong and Yeongsan River, Korea: The relevance to blooms of blue-green algae

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Nakdong (ND) and Yeonsan (YS) river, two of 4 major rivers in Korea, are suffered from blooms of blue-green algae in hot season. Interestingly, YS river with greater nutrient levels(i.e., higher nitrogen/phophate concentration) shows less population of blue-green algae, indicating unknown factors affecting the algal growth/blooms. As the suspected reasons (or regulators), the occurrence and concentration of pesticides including herbicides and fungicides was investigated using the state-of-the art analytical method based on multilayer-SPE-LC-HRMS tool. The sampling campaign was undertaken parallel for both river from June to October, 2016. Prior pesticides identified in both river include 2 Fungicides(Iprobenfos, Tebuconazole), 5 Herbicides(Alachlor, Diuron, Mefenacet, Oxadiazon, Simazine), 2 Insecticides(Chlorpyrifos, Diazinon). The median concentrations of pesticides in Nakdong/Yeongsan rivers are as follow: Iprobenfos 178/382 ng/L, Tebuconazole 24/38 ng/L, Alachlor 49/171 ng/L, Diuron 22/63 ng/L, Mefenacet 142/290 ng/L, Oxadiazon 75/578 ng/L, Simazine 7/5 ng/L, Chlorpyrifos 178/382 ng/L, Diazinon 5/7 ng/L. The Sum of the median value in YS(1,755 ng/L) river is about 3 times greater than that of ND river(597 ng/L). Although integrated effects of identified pesticide cocktail on algal growth is not well estimated, the signifcant difference in the occurrence frequency and the concentration of pesticides can play a role as a regulator for blooms of blue-green algae.

MO027

Spatial and temporal relationships reveal the impact of dams on river ecosystem resilience: The Ebro river (NE Spain) as case study

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River ecosystems are exposed to continuous changes in hydrological conditions, nutrients inputs or organic contaminants, among other stressors, that are reflected on their longitudinal pattern characteristics. However, this continuity might become severely perturbed by the existence of large reservoirs that causes ecological and hydrological alterations, as in the case of Ebro river (NE Spain). River ecosystems can be described as networks encompassing both local dynamics and neighbor interactions involving chemical, physical, biological and geomorphological processes. The present work uses the river network structure and so, considers both the spatial and temporal relationships of biological and other environmental descriptors associated to each river stretch. With this approach, we have integrated environmental, chemical and biological data, monitored in the Ebro river mainstream between Zaragoza and the river mouth. The biological descriptors measured are chlorophyll-a, alkaline phosphatase activity and number of phytoplankton species (diversity). The environmental variables considered were those reflecting anthropogenic pressures (temperature, conductivity, pH, dissolved oxygen, nitrogen, total phosphorus, dissolved organic carbon). River flow was measured by the local water authority. Data were examined by means of lag-1 autocorrelations, Principal Component Analysis and Normal Modes Analysis. Its application enables a first interpretation of the longitudinal patterns and connectivity in the Ebro. Results presented here aim to better understand (a) the relevance of spatial discountinuities on the river ecosystem associated to the presence of dams (b) the relative contribution of synchronization vs. local dynamics acting on the biological components in each monitoring campaign (c) the possible occurrence of early warning signals of system resilience changes associated to such discontinuities. *Acknowledgements* - This work is supported by the Spanish Ministry of Economy and Competitiveness through the Redes de Excelencia project NET-SCARCE (CTM2015-69780-REDC). The research leading to these results has received funding from the European Comunities 7th Framework Programme under Grant Agreement No. 603629-ENV-2013-6.2.1-Globaqua. The authors wish to acknowledge the Confederación Hidrográfica del Ebro for providing the data used in this study.

Polar ecotoxicology: hot issues in cold climates (P)

MO028

Toxicity of hexachlorobenzene to humpback whale cells - establishment of a passive dosing assay and in vitro toxicity testing

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Persistent Organic Pollutants (POPs) are distributed globally through long-range atmospheric transport and finally accumulate in polar regions, where their revolatilisation is restricted by temperature limitations. One of the POPs found in the highest concentrations in Antarctica is hexachlorobenzene (HCB). Due to its hydrophobicity (log Kow = 5.5) it accumulates in biota and biomagnifies in the food chain, leading to a greater risk for species at higher trophic levels. Humpback whales (*Megaptera novaeangliae*) feeding in Antarctica have been found to accumulate large amounts of HCB in their adipose tissue. During their long migration they undergo extended periods of voluntary fasting, whereby their lipid reserves decrease. As a consequence, accumulated POPs, including HCB, increase substantially in concentration and are remobilised, leading to re-exposure of individuals. However, toxicological risk assessments for these large marine mammals are difficult. In this work, a cell line of humpback whale fibroblasts was employed to assess the toxic effects of HCB *in vitro*. The challenge in this approach lies in HCB's hydrophobicity and volatility; losses by sorption and evaporation lead to unstable exposure concentrations, potentially resulting in underestimation of its toxicity. To overcome this issue, a passive dosing setup for HCB was established. Silicone O-rings were used as a reservoir for dosing HCB to the cell culture medium

DMEM/F12. The partitioning coefficients of HCB from a loading buffer of methanol/water to silicone, and from silicone to DMEM/F12 were determined, and the stability of concentration over time was verified. Partitioning of HCB to DMEM/F12 containing 1% fetal bovine serum (FBS) was significantly higher compared to FBS-free medium, with approximately five times higher concentrations. Viability of humpback whale cells exposed to HCB was assessed using fluorescent dyes as indicators of metabolic activity (Alamar blue) and membrane integrity (CFDA-AM). Exposure of cells in confluent monolayers revealed no effect of HCB on cell viability. However, cells exposed to HCB during the phase of suspension showed significantly reduced cell attachment. This suggests that HCB exerts toxicity on humpback whale cells when combined with additional stressors such as suspension state. These results may indicate higher sensitivity of humpback whales to chemical stress when they are affected by multiple stressors.

MO029

Methylmercury in Arctic Seabirds - Seasonal inter-tissue differences

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Mercury (Hg) is a natural element, however, anthropogenic activities have increased its release and dispersal in the environment. Microorganisms may convert elemental Hg to organic methylmercury (MeHg), which is the most toxic form of Hg, and has a greater potential for bioaccumulation than elemental Hg. Arctic marine birds may be at risk from increasing Hg levels associated with changing climatic conditions and continuous Hg deposition. The main objective of this study was to quantify the accumulation of MeHg in black-legged kittiwake (*Rissa tridactyla*) and common eider (*Somateria mollissima*) from Svalbard, Norway, focusing on possible seasonal changes in MeHg accumulation, as well as possible differences between bird tissues. We also quantified the relationship between total Hg (TotHg) and MeHg, as well as between TotHg and selenium (Se) in the different species and tissues, to better understand Hg dynamics and the role of Se in Hg detoxification, respectively. Seasonal changes in MeHg, TotHg and Se accumulation, as well as differences between bird tissues, were observed. The seasonal differences in Hg concentrations appeared as conspicuous as geographical differences, e.g. from birds of the Canadian Arctic. MeHg, TotHg and Se all showed declining concentrations from May to October in both species. However, the decrease was not as prominent for Se, as for TotHg.

MO030

Effect of remoteness and global fractionation on legacy contaminants in a top predator seabird across the Northeast Atlantic

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Long-range transport has resulted in spatial gradients of persistent organic pollutants (POPs) in the environment. Examples are latitudinal gradients due to global fractionation processes or with increasing remoteness from POP sources. The occurrence of organochlorines (OCs) and polybrominated diphenyl ether (PBDEs) was analysed in 204 great skuas (*Stercorarius skua*), a top predator seabird, from different colonies across the North-Atlantic from Shetland to Svalbard to determine if contaminant occurrence was explained by long-range transport (global fractionation or remoteness gradients) and/or by ecological factors such as diet. The sum contaminant levels in plasma ranged across two orders of magnitude, from 40 to 7600 ng/g wet weight in the North-Atlantic great skuas colonies, and differed significantly across colonies. Contrary to the expected spatial gradient, the second most remote colony located at the second highest latitude (Bjørnøya), had the highest contaminant concentrations. The relative pattern also differed among colonies; whilst Shetland great skuas having a slightly higher proportion of heavier and less volatile high chlorinated PCB congeners than colonies further north, as expected from the global fractionation theory, no clear latitudinal or remoteness gradient was evident. Thus, the variation in contaminant occurrence in great skua in the North Atlantic colonies did not reflect long-range transport through a latitudinal or remoteness gradient. Variations in individual behaviour of great skuas' migration strategies and diet sources, rather than long-range transport, may explain their contaminant occurrence.

Wildlife ecotoxicology: cumulative effects through the food chain to the community (P)

MO031

Historical mercury contamination in Belgian rivers; screening for suitable biomarker genes of the three-spined stickleback (Gasterosteus aculeatus).

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Historical metal contamination of the Belgian surface waters dates back to the nineteenth century. In recent decades, regulatory implementations and increased sanitation efforts did contribute to an overall better water quality. Yet, traces of past contamination events are still detectable. Amongst others, there is the presence of mercury in aquatic sediments and biota, a reminder of historical metal smelting activities. The overall goal of this project is to discover whether there is a significant inheritability of the adaptive traits of populations towards the presence of sub-lethal mercury concentrations. The three-spined stickleback, *Gasterosteus aculeatus*, has been selected as a sentinel species for this research because of its wide dispersion, and tolerance towards contaminated environments. During a first field campaign, two rivers known for their historical mercury contamination and one near-to-pristine stream, were sampled at four different trophic levels; water, sediment, invertebrates and fish. All samples were subjected to an optimized protocol for the determination of the exact concentration of the following metals; As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn. First, we wanted to have an estimation of the metal concentration which is still present at these four levels of the food web. These results were then used to calculate site-specific bioaccumulation and biomagnification factors for the analyzed metals. In addition, some of the stickleback caught at each site were dissected and their liver was collected. A set of biomarkers for oxidative stress was selected and used to evaluate the effect of contamination in each river at a molecular level. Therefore, the expression of the following genes was determined; *catalase*, *metallothionein*, *heat shock protein 70*, *glutathione peroxidase* and *superoxide dismutase.* Novel primer pairs were developed, and have now been evaluated suitable for real time qPCR analysis. First results suggest an overall higher expression of catalase in comparison to the four other genes of interest, while the difference among sites was less pronounced.

MO032

A little bird once told me: Health affects attributed to NO2 exposure in urban areas could be from confounding co-contaminants

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Epidemiological studies have identified relationships between numerous disease syndromes in humans, air pollutants such as oxides of nitrogen (NO_x), and more recently, sulphur and fine (respirable) particulate matter. Motor vehicles are the major source of NO_x in the USA, therefore, in an effort improve air quality, environmental regulators such as the US EPA have set increasingly stringent standards for vehicle emissions and fuel quality. Since 1990, in the USA total NO_x emissions have nearly halved and hydrocarbons (such as benzene, toluene, ethylbenzene and xylenes, BTEX) measured at the exhaust pipes of small vehicles have decreased almost 10-fold between 1993/4 to 2014. It is very challenging, using either field or epidemiological data, to distinguish among the health effects of highly correlated pollutants, particularly when they are co-emitted from mobile sources. We propose that while NO_2 may have been featured as the major contributor to negative health effects in the past, the dramatic reduction in NO_2 concentrations may reveal that the other products of fossil-fuel combustion such as benzene or toluene, are emerging as important contributors. Despite many epidemiological studies into the health effects of exposure to urban air pollution on human populations, there is very little information on the effects of realistic concentrations of pollutant mixtures on wildlife, from field or experimental studies. The current investigation was designed in two parts to address this deficit: Part 1 examined the effects of urban air pollution on nestling European starlings (*Sturnus vulgaris*) in the city of Calgary, Alberta, Canada; while Part 2 exposed wild-caught, adult starlings to vehicle exhaust for 5 hours per day, 6 days per week for just under one month. We shall compare and contrast the exposure to contaminant mixtures found around the urban environment with those obtained in the experimental exposure, and highlight biological responses (biomarkers) in the birds that tie together these studies. The results of those investigations will be evaluated against those of a similar study published in 2016, suggesting that BTEX, not NO_2, pose the larger responsible for the observed toxicological responses.

MO033

Can autopsy measurements be used as health indices to provide early warning of population impacts?

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A key challenge when assessing the impacts of contaminants, and deciding whether there is a need to mitigate against them, is to evaluate if populations effects are likely to occur. Biochemical and physiological biomarkers may be too sensitive in that they do not translate into ecologically significant effects on individuals or populations, but delaying mitigation until populations are impacted is too late - population recovery may take decades, if it happens at all. Early warning of impacts on population processes is needed. In the UK, the Predatory Bird Monitoring

Scheme (PBMS; http://pbms.ceh.ac.uk/) is a national-scale scheme used to detect and quantify current and emerging chemical threats to the environment and in particular to vertebrate wildlife. We autopsy approximately 300 and 400 predatory bird carcasses per year and make some 60 macroscopic observations and measurements on each. The information gathered can be used to monitor the health status of the birds at the time of their death or at a particular stage of their development. Such measurements, here termed population health indices, can be linked to measures of contaminant exposure and potentially provide early warning of impacts on processes that ultimately may affect populations. We examined the use of post-mortem measurements as potential population health indicators in a candidate species, the sparrowhawk, *Accipiter nisus.* We broadly categorised different measures as indicators of change in: (i) population demography because of altered recruitment, survival and mortality (measures were sex ratio, proportion of first-year birds, proportion deaths from starvation or disease, eggshell index); (ii) nutritional status (measures were body weight, fat score, condition index) and (iii) physiological stress (fluctuating asymmetry). We were able to track changes in some of these indices as sparrowhawk populations recovered from exposure to organochlorine pesticides, and established current "norms" against which statistically-significant future change can be quickly detected. We consider how such health indices can be reported in real-time, extended across different trophic pathways and ecosystems, and detect nascent ecologically-significant changes. We argue that rapid detection of such changes, coupled with contaminant monitoring and allied studies, could prove a trigger for timely and targeted iterventions/mitigation.

MO034

Blood concentrations of organochlorine compounds in an avian predator endemic to southern Africa: associations with habitat, electric transformers and diet

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Persistent pollutants such as organochlorine compounds (OCs) have been highlighted as a cause of population decline in apex predators, and understanding the patterns of OCs contamination can be crucial for the conservation of affected species. Despite the large number of studies, little is known on the threats to African raptor species. Here we report on OC concentrations in an endangered top-predator endemic to southern Africa, the Black Harrier *Circus maurus*. Blood samples were collected in 2012-2014 from wild nestlings (n=90) and adults (n=23) in south-western South Africa, a region where agriculture and urbanization have rapidly developed since the 1950s. Levels of polychlorinated biphenyl (ΣPCB) and dichlorodiphenyltrichloroethane (ΣDDT, for p,p'-DDT + p,p'-DDE) were detected in 79% and 84% of sampled individuals respectively, with varying concentrations among demographic groups. Nestlings had significantly higher ΣPCB and p,p'-DDT concentrations than adults, which in contrast presented higher levels of p,p'-DDE than nestlings. Levels of ΣPCB significantly increased with "Transformer Density Index", an innovative index assessing number and power of electricity transformers around active nests, which we propose as a useful tool for assessing ΣPCB exposure in wildlife. Levels of p,p'-DDE significantly increased with the proportion of wetlands within the breeding territory. No association was found between OC levels and the protected areas status of nesting sites. Furthermore, p,p'-DDE levels significantly increased with higher percentage of bird biomass in an individual's diet, confirming the intra-specific relation between diet composition and OCs contamination. Finally, we show that OCs had sub-lethal effects on indicators of health condition. White blood cell count increased with higher p,p'-DDT levels, showing a reaction by the immune system. The Heterophils:Lymphocytes ratio increased with higher ΣPCB levels, suggesting increased physiological stress and reduced immunity. We suggest that detection of OCs in Black Harriers highlight a current threat to other sympatric predators.

MO035

Blood levels of heavy metals and arsenic in black-necked grebe Podiceps nigricollis during the moulting period in the Odiel Marshes, SW Spain

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Black-necked grebe (*Podiceps nigricollis*) has in the salt pans of Odiel Marshes (SW Spain) one of the most important moulting areas in Western Europe. However, Odiel Marshes are impacted by the mining and industrial activities located in the Odiel and Tinto River Basin and the city of Huelva, respectively. Grebes moulting in this wetland could be therefore exposed to heavy metals and metalloids during a critical period, such is moulting, when energy and protein requirements are elevated. Here we have studied the hypothesis that black-necked grebes would experience an increase in blood levels of heavy metals and metalloids during their stay at Odiel Marshes, between August and December. Alternatively, we also consider the hypothesis that grebes could reduce the concentration of some elements by excretion with the newly grown feathers. Blood levels of arsenic, lead, cadmium, mercury, copper and zinc were measured in blood of 180 black-necked grebes captured for a ringing program of the Doñana Biological Station. We studied the effect of sex, age (1styear, 2nd year and adults), moulting status (old plumage, active moult or new plumage), scaled mass index and date on the measured levels of metal(loids). Most of the studied elements showed significant temporal variations that could be associated with moulting status or with the time spent at the Odiel Marshes. Arsenic levels decreased during active moult (225 ng/ml) but increased afterwards to much higher blood concentrations during the stay at Odiel Marshes (488 ng/ml). Blood Pb levels were highest at the end of active moult (October: 18 ng/ml) and showed a slight increase with the age of birds. The analysis of recaptured birds in the same year also revealed an increase in blood Pb levels from October to November. One remarkable outlier with 404 ng/ml may correspond to the ingestion of a lead shot or a fishing weight. Mercury levels were lower in birds with new feathers (256 ng/ml) than in those with old feathers (303 ng/ml). Three birds showed extremely high levels of mercury (>10 μg/ml). Copper and zinc levels in blood were highest in birds with active moult. Our results show significant temporal variations of metal(loid) levels that can be associated with the requirements for feather formation (essential elements such as Cu and Zn) or with a detoxification due to the transfer of elements from blood to feathers (Hg).

MO036

Sublethal effects of neonicotinoids on farmland birds

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More intensive farming requires ever increasing amounts of pesticides to maintain productivity. The sustainable use of pesticides is vital if we are to maintain effective ecosystem services. Evidence is accumulating that the widespread usage of NN insecticides is having a variety of unexpected impacts on non-target organisms and ecosystem services. Neonicotinoids (NNs), the world's most widely used insecticides are suggested to harm bees and other pollinating insects. This has led to a two-year EU moratorium on three NN products began at the end of 2013. However, NNs are still used as a seed dressing for crops such as cereals and beet. Recent research has suggested a link between the use of NNs and declines in numbers of farmland birds. However, the research only looks at associations at a large spatial scale with no evidence for the mechanisms involved. Direct toxic effects on birds are possible via the consumption of feeds coated with NNs and/or consumption of plants or insects that have taken up NNs. This might result in death or reduced reproduction but also non-lethal effects such as changes in health, foraging behaviour that can affect fitness. This research will integrate field, lab and aviary studies to determine whether NNs can move through the food chain exposing birds to lethal or sub-lethal concentrations. In addition, by experimentally manipulating exposure of birds to NNs, we hope to determine whether NNs cause changes in the behaviour, physiology and overall health of birds.

MO037

Embedded lead-shots as a potential source of lead exposure in birds of prey

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Lead hazard for birds of prey hunting far away from wetlands has been well documented. Examples include the California condor (*Gymnogyps californianus*) (Janssen et al., 1986), the Turkey vulture (*Cathartes aura*) (Kelly and Johnson, 2011), the Eurasian griffon vulture (*Gyps fulvus*) (Garcia-Fernandez et al., 2005) or the Red kite (*Milvus milvus*), (Pain et al., 2007). The suspected origin of lead for these populaiton is bullet fragmentation from big game hunting and ingestion of lead residues by birds of prey. Stable isotopes analyses have allowed several authors to identify lead ammunition as the major source of lead exposure for these birds (Pain et al., 2007 ; Berny et al., 2015, Madry et al., 2015). Less is known, however, about the diffusion of lead from embedded ammunitions I healed wounds of shot birds of prey. The purpose of the present study was to investigate the blood lead concentration in birds of prey admitted to bird rescue centers

MO038

Contamination status by persistent organic pollutants of the Atlantic spotted dolphin (Stenella frontalis) at the metapopulation level

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The Atlantic spotted dolphin (*Stenella frontalis,* ASD) is an endemic species of the tropical temperate Atlantic Ocean, with a widespread distribution. Although this species has been the subject of a large number of studies throughout its range, this species remains in the "data deficient" category of the International Union for

Conservation of Nature (IUCN). Chemical pollution by persistent organic pollutant is one of the major threats this species faced but have paid little attention until today. Thereby, the objective of this study is to investigate the contamination status of this species at the metapopulation level and investigate the spatial and temporal variation of POP concentrations and accumulation. To this aim 116 samples of blubber were analysed for a wide range of POP classes (PCBs, DDTs, PBDEs, HCHs, chlordanes, HCB and mirex). Inter-location differences on POP concentrations and patterns were clearly evidenced, allowing to identify Canary Islands and Sao Paulo as "POP hotspots" for this species. Moreover, sex of the animals and the year when they were sampled or captured revealed an important effect on the majority of the POP classes analysed. Comparison with toxicity thresholds available in the literature showed that the concentrations found in ASD are not alarming. However, the combined effect of chemical contaminants presented in the environment should not be overlooked. In our knowledge this is the first study aiming to assess the contamination status by POPs of ASD at the metapopulation level contributing with important data that could be considered in future local conservational programs.

Advances in the Environmental Fate of Down-the-Drain Chemicals, including Pharmaceuticals (P)

MO039

Uptake of emerging contaminants under hydroponic condition

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Many emerging contaminants that enter wastewater treatment facilities are incompletely removed ending up in the final effluent or in the resulting sewage sludge. When this effluent or sewage sludge is used as reclaimed water for agronomical purposes, contaminants present in the matrix can be mobilized to crops by the interaction between the soil and the plant roots. Assuming their stability, the affinity of emerging contaminants for different crops depends on several factors, mainly the hydrophobic partition coefficient (Kow), the acid dissociation constant (pKa), the soil pH, and the soil organic carbon-water partitioning coefficient (Koc).Thus, predicting the uptake of organic chemicals by roots and their translocation to other parts of the plant is of significant value for conducting a proper risk assessment. However, the mechanisms involved in plant uptake remain poorly understood so that there is still a lack of information on translocation behavior of waterborne contaminants into the different parts of the plant The use of hydroponics allows the production of plants by eliminating environmental factors that may decrease the experimental reproducibility. Using a continuous flow solution culture system we report data on the accumulation of atenolol (AT), carbamazepine (CBZ) and triclosan (TCS) in different parts of these plants of economic interest; i.e. radish (Raphanus sativus), lettuce (Lactuca sativa) and tomato (Solanum lycopersicum). Seedlings (7-10 d old, with two cotyledons and approximately 2 cm root length) were transferred to hydroponic systems containing the nutrient solutions spiked with the different contaminants. After 21 days, plants were collected, and AT, CBZ and TCS content were analytically determined in the different parts of the plants; i.e. roots and shoots. This work was possible thanks to Spanish Ministry of Economy and Competiveness projects CTM2013-44986-R and CTM2014-52388-R.

MO040

Assessment of sources, occurrence and fate of the fungicide carbendazim in water

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The fungicide carbendazim is used in agriculture to protect plants and fruits against fungi but it is also a common agent used to protect outdoor surfaces such as wood fences or facades of buildings. Therefore, carbendazim can be washed off during rain events and then detected in wastewater and surface water. However, some studies report the occurrence of carbendazim also during dry periods. This suggests an unrecognized input source of the fungicide in the environment. Therefore, this study aims at assessing the occurrence of carbendazin in wastewater effluents from Germany during dry weather periods. Paper and textiles will be investigated as potential input sources. In addition, possibly occurring TPs will be generated and characterized by electrochemical degradation. The analysis of wastewater effluents collected during a dry weather period from 22 municipal treatment plants revealed the occurrence of carbendazim in 100% of the samples with an average concentration of 25 ng/L. A laboratory-scale leaching test confirmed the release of carbendazim from textiles (T-shirts and table cloth) and paper (newspaper, kitchen sorbing paper and toilet paper). Moreover, the hypothesis of paper as a source of carbendazim was further sustained by samples collected in small rivers flowing next to 2 paper industries which showed an increase in the concentration of carbendazim immediately downstream of the plants. A back-of-the-envelope calculation using the amount of toilet paper used and the wastewater volume discharged in Germany showed that carbendazim input via toilet paper alone can explain a concentration in wastewater of 2 ng/L. It can be increased to about 5 ng/L during dry weather discharge. The bench-scale electrochemical degradation of carbendazim and sample analysis by high- resolution mass spectrometry revealed the formation of seven transformation products. The main four transformation products could be identified by high-resolution tandem mass spectrometry. For the other three minor TPs chemical formulae could be tentatively assigned based on their accurate masses. Finally, the major transformation products of carbendazim have been also detected in a major stream. Bench-scale electrochemical degradation experiments combined with high-resolution mass spectrometry turned out to be a successful approach to investigate the occurrence and fate of major transformation products of carbendazim.

MO041

Biodegradation of 3 pharmaceutical compounds in activated sludge

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Different tests were carried out to determine the biodegradability of three pharmaceutical compounds (PCs), one anti-epileptic drug (carbamazepine), an anti-inflammatory compound (diclofenac) and the a steroid and estrogen sex hormone (17β-estradiol). PCs, the mineralization tests were based on OECD guideline 310 (Ready Biodegradability - CO_2 in sealed vessels (Headspace Test) 2006) and the testing period was 28 days, with CO_2 measurements being carried out every 3-4 days using gas chromatography. The tests were inoculated with secondary sewage sludge from the Amsterdam West WWTP which has a biological capacity of 1.0 million population equivalents. The tests included controls to measure the abiotic degradation of the PCs and to test for any inhibition of the inoculum by the tested chemicals. Additional tests for LC-MS analyses were similar to those used in the mineralization experiments. 1 mL aliquots were extracted from the sealed vessels every 3-4 days for a period of 28 days. These were filtered to remove particles and analyzed using an LC-MS/MS system. Most of the toxicity tests showed no inhibition of microbial activity. Nevertheless, no removal of mineralization of carbamazepine and diclofenac were found, consistent with the known persistence of these molecules. However, 17β-estradiol showed some degradation with approximately 10% removal after 28 days. These results indicate that aquatic ecosystem near the sewage discharge site are probably exposed to these pharmaceutical compounds.

MO042

Psychiatric drugs bioaccumulation, metabolization and depuration in bivalves under climate change conditions

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Psychiatric drugs have been found in different water bodies, including coastal areas. These compounds are designed to be pharmacologically active in humans. Therefore due to the conservation of metabolic pathways within the organisms, psychiatric drugs can have an effect on non-target organisms like bivalves. Furthermore, bivalves are highly consumed species; thus, their potential bioaccumulation of contaminants may represent a risk for consumers. Besides, the expected climate change effects on seawater (warming and acidification) may alter the behavior of these compounds in the organisms, including their bioaccumulation, metabolization and depuration. In this study, mussels (*Mytilus galloprovincialis*) were exposed to a mix of three psychiatric drugs: venlafaxine, carbamazepine and citalopram under controlled conditions. The bioaccumulation and depuration of these compounds were evaluated. In addition, the formation of venlafaxine and carbamazepine metabolites was also studied. The experiment lasted 40 days, where mussels were exposed in the first 20 days to a mix of contaminants at 10 μg/L, whereas no contaminants were added in the last 20 days. Climate change was simulated in a recirculation system by exposing organisms to water acidification and warming conditions. Results showed clear bioaccumulation for all compounds, being the bioaccumulation factors: 1648 Liter/Kilogram (L/Kg) for citalopram, 341 L/Kg for venlafaxine and 29 L/Kg for carbamazepine (mean of all spiking treatments). The bioaccumulation varied mostly between compounds, but some differences were also registered due to the treatments. For instance, warming seems to increase the bioaccumulation of carbamazepine, while acidification decreases venlafaxine and citalopram levels in mussels. Only venlafaxine showed metabolization, with three metabolites being detected (O-desmethylvenlafaxine, N-desmethylvenlafaxine and NO-didesmethylvenlafaxine) at levels ranging from non-detected to 100 ng/g. The expected climate change effects, water acidification and warming, seems to decrease the metabolization of venlafaxine in mussels. Finally, results showed a high percentage of depuration after 20 days of clearance, ranging from 51% to 84% considering the three spiked compounds. However, none of the compounds was completely eliminated after this period, indicating that 20 days of depuration is not sufficient to eliminate these contaminants from mussels.

MO043

Transformation of diazepam and related benzodiazepines during chlorination

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Benzodiazepines are the most prescribed pharmaceuticals in the treatment of the anxiety, depresion, convulsion, insomnia or panic disorder, being Diazepam one of most prescribed. In the same way as other pharmaceuticals, these compounds can enter watewater treatment plants (WWTP) and, if they are not completely removed, they can reach the surface water, which is finally a source of drinking water [1]. A common step in the drinking water production is the disinfection by using e.g. chlorine, ozone. However, rections between disinfectant agents and organic contaminants can ocurr generanting a range of transformation products (TPs) which are in some cases more toxics than precursor compounds [2]. It's worth mentioning that the study of the reaction of chlorine with benzodiazepines has not been performed so far. The aim of this work was to study the reaction between chlorine and diazepam and three related compounds (nordazepam, oxazepam and temazepam) as well as the identification of their TPs using liquid chromatography (LC) followed by high resolution mass spectrometry (HRMS) with a quadrupole-time of flight spectrometer (QTOF). For those benzdiazepines which showed a significant degradation, i.e. diazepam, oxazepam and nordazepam, parameters affecting to the degradation reaction (pH, chlorine and bromide content) were studied in detail using an experimental design. Moreover, 9 TPs were tentatively identified. The transformation of the studied benzodiazepines led mainly to the formation of benzophenone or quinazoline derivates. Finally, the evaluation of their ecotoxicity was carried out using quantitative structure-activity relationship (QSAR) software tools: Ecological Strucuture Activity Relationship (ECOSAR) and Toxicity Estimation Software Tools (TEST), where it was observed that some TPs are of higher concern than precursor drugs. **Bibliography:** [1] T. Kosjek et al. Water Res 6 (2011) 355–368. [2] C. Postigo J Hazard Mater 279 (2014) 461–475. **Acknowledgement**: The authors acknowledge the support of Xunta de Galicia (EM2014-004, GRC2013-020 and I.C. her postdoctoral grant) and FEDER/ERDF.

MO044

Comparison of two cell lines to select an effective method for the simultaneous determination of phototoxic and photogenotoxic potential of pharmaceutical pollutants.

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Light is an important factor that can modify the impact of pharmaceutical pollutants or industrial and personal care products waste chemicals on the environment. Many of these compounds after solar irradiation generate photoproducts that could be significantly more toxic and genotoxice than the initial chemicals. Evaluation of this risk is especially important for compounds that may persist in the environment which may result in a chronic exposure of humans and other living organisms. The assessment of possible phototoxic and photogenotoxic abilities is also relevant to evaluation of the safety of photoproducts obtained by the degradation of pharmaceutical pollutants with UV in waste water treatment plants. One of the methods proposed by the OECD to evaluate the phototoxicity of chemicals is the *in vitro* neutral red uptake assay with the BALB/c 3T3 cell line. However, to verify the photogenotoxicity of tested compounds an additional test should be performed. According to reports one of the techniques suitable for this purpose is the micronucleus test. The mammalian V79 fibroblasts are among cell lines recommended for this assay instead of the BALB/c 3T3 cells. Conducting micronucleus assay requires additional evaluation of the toxicity and phototoxicity of tested substance for V79 cells. Assessment of the phototoxicity and photogenotoxicity could be more effective if performed simultaneously with only one cell line. In this project we compared the results of the neutral red uptake assay obtained for V79 cells and BALB/c 3T3 cells. The cell lines were exposed to a number of compounds with documented different phototoxic abilities, including: nonsteroidal anti-inflammatory drugs, several fluoroquinolones, tetracycline and chlorpromazine as a positive control. Irradiation of samples was performed in microplates in the sunlight simulator: SUNTEST CPS+. The neutral red uptake assay was prepared on the basis of the OECD 432 guideline that enabled to compare obtained results by the calculation of the Photo-Irritation-Factor and the Mean Photo Effect of each tested compound. The statistical analysis of these data allowed to verify the hypothesis of comparable sensitivity of both cell lines. The project was financed by the Medical University of Warsaw from the Grant for Young Scientists managed by Anna Zgadzaj (FW14/PM2/16).

MO045

Antibody Mimetics for the Detection of Environmental Contaminants

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Conventional immunoassays rely on antibodies which provide high specificity against a target analyte; however, they can be severely limited when it comes to detecting small-sized, non-immunogenic targets, such as pharmaceuticals or other emerging environmental contaminants. Recent advances in protein engineering have led to the emergence of antibody mimetics (AMs) that offer significant advantages over traditional antibodies, including reduced batch-to-batch variability, high stability and *in vitro* selection to ensure rapid discovery of binders against a wide range of targets. Affimers[1] are a recent example of AMs; inspired by the versatility of this technology we aim to explore the potential of Affimers as suitable bio-receptors for the detection of environmental contaminants, such as methylene blue and diclofenac. A variety of techniques were used, including ELISAs, cyclic voltammetry (CV) and piezoelectric (QCM-D), in order to characterise the affinity of Affimers, develop an approach of an Affimer-based competition assay, and finally, evaluate Affimer binding performance in the increased complexity of surface water samples. 1. Tiede, C.; Tang, A. A. S.; Deacon, S. E.; Mandal, U.; Nettleship, J. E.; Owen, R. L.; George, S. E..; Harrison, D. J.; Owens, R. J.; Tomlinson, D. C.; McPherson, M. J. Adhiron: a stable and versatile peptide display scaffold for molecular recognition applications. *Protein Eng Des Sel* **2014**, 27, 145-155.

MO046

Identification of micropollutants in surface water originating from sewage treatment facilities by using target and suspect screeening strategies based on regulatory databases

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Discharges from sewage treatment facilities are a major pathway of organic micropollutants to the aquatic environment. It includes conventional wastewater treatment plants (WWTP) and also small-scale on-site facilites (OSSFs). OSSFs are widespread in sparsely populated areas, where the establishment of conventional WWTPs for the sanitation of wastewater is not feasible. The main objective of the present study is to compare the efficiencies in the removal of micropollutants between WWTPs and OSSFs and evaluate the impact of these facilities in the receiving aquatic environment. The present work used a target list of 80 organic micropollutants including pharmaceuticals, personal care products, pesticides or perfluoroalkyl substances (PFASs), among others. 60 out of the 80 target substances were detected in the evaluated wastewater samples. The studied facilities showed a varying pattern of removal efficiencies. Some substances showed high removal efficiency (e.g. parabens), while other substances showed no significant removal (e.g. diclofenac). Overall, the OSSFs showed similar efficient performance than medium or large scale conventional WWTPs. The concentrations found in the receiving surface water samples show that water quality is directly related to the presence of OSSFs and WWTPs. In all cases, both the number of compounds as the concentration of these increased significantly after the input of a sanitation facility. However, the existing target analysis methodologies only allow the detection of a very small fraction of the substances present in environmental samples. The application of suspect screening, with a suspected screening list based on prior information but with no reference standard, greatly increases the list of substances that can be identified. A suspect list was built based on the hypothesis that regulatory databases can assist in the prioritization of potentially relevant substances. Therefore, we used the Swedish Chemical Agency database to prioritize substances present in wastewater effluents and surface water by using different criteria including (i) the occurrence on the market, (ii) the consumed tonnage or (iii) the use pattern. The final suspect screening list contained ≈150 organic micropollutants (high ratio of industrial chemicals) and was used to identify organic micropollutants in wastewater and surface water. For most of the selected compounds there was a lack of literature regarding their presence in the aquatic environment.

MO047

Identification of micropollutants in sewage effluent and receiving seawater in Arctic region

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This study is aimed at investigating the contamination of micropollutants (e.g., pharmaceuticals and personal care products) in Arctic region as the consequence of sewage discharge(9ton per day) from Ny-Alesund research town, Svalbard islands. For chemical analysis, the effluent samples and the receiving seawater around the drainage were collected in parallel for 3 consecutive days in July 2016. The samples (250mL effluent, 2L seawater) were pre-treated via solid phase extraction before target/suspect/nontarget screening using LC-ESI-Orbitrap(Q Exactive plus). As the results, pharmaceuticals, personal care products(PCP) and pesticides were detected in every effluent samples. The identified pharmaceuticals include antipyrine, cetirizine, metoprolol, naproxen, diclofenac, and ephedrine. One transformation of PCP and pesticide, galaxolidone and diethly toluamide were also detected. In seawater samples, caffeine, cetirizine, and pyridine were detected in SL1 located close to the drainage, indicating those compounds are persistent in the area among other identified pollutants. On the other hand, diethyl toluamide, caffeine and galaxolidone were detected in SL2 and SL3 which are relatively far located from drainage, indicating wide distribution of those micropollutants.

MO048

Emission of synthetic musk fragrances in surface waters during rain events in urban environments

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Synthetic musks are widely used as fragrances additives in many consumer products, they are widely present in the environment and they tend to accumulate in sediment, sludge and biota. Their main emission sources are the discharges of urban wastewater treatment plants (WWTPs), where the musk fragrances are partially removed both by sludge adsorption and biological oxidation. The fragrances most commonly found in surface waters are galaxolide and tonalide and the former is transformed by oxidation in its main transformation product, galaxolide lactone. We developed and tested an Isotope Dilution GC-MS method for the analysis of synthetic musk fragrances in waste and surface waters. The method, validated taking carefully into account the problem of blank contamination, allows to determine 9 compounds, including nitro-, polycyclic-, macrocyclic musks and a transformation product (galaxolide lactone), which can be a specific tracer of WWTP discharge. Compounds are measured on whole water after separation of dissolved and particulate phases. The occurrence of this class of substances has been evaluated in river Lambro, a tributary of river Po and in its basin, which is an area with a very high pressure from population, industry and intensive agriculture. Monitoring campaigns on river water have been carried out in dry and rain periods in order to study the dynamic of musk concentrations under different hydrological conditions. Hydrological and physico-chemical variables as well as caffeine have been continuously measured during the rain events in order to put into correlations musk concentrations with water sources such as run-off and the combined sewer overflows (CSOs). The combined study of the evolution of whole water and SPM concentrations together with the ratio between galaxolide and its transformation products (galaxolide lactone) allowed to discriminate among the different sources. In fact at the beginning of the rain event, the galaxolide concentration in whole water increases, but the galaxolide lactone to galaxolide ratio, which can be used as a specific tracer of the oxidative removal processes in WWTPs, decreased, showing the contribution of not treated sewage waters to the pollution load in rivers. The concentrations in SPM also changed during the course of the event, and can help to understand the presence of different water sources in the basin.

MO049

Source determination of endocrine activity in the Zenne River, Brussels. Combining flux modelling with in vitro endocrine activity measurements

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Increasing awareness concerning endocrine disrupting chemicals (EDCs) and endocrine active chemicals (EACs) in waterways is prevalent as evidenced by the WFD (2013/39/EU) who prioritized some estrogenic chemicals into their watch list and recommended to EU member states to monitor these substances. During our study we combined water fluxes with EDC distributions in the Zenne River, Brussels, to obtain a mass budget of the pollutant load and track the (highly dynamic) interaction along the river to assess sources and sinks otherwise hidden by classical discrete sampling. This pollutant load was based on the total endocrine activity following in vitro reporter gene analyses, rather than at the individual compound level. Samples were collected from the river (4 locations), in the WWTPs (effluent and influent), and from hospital effluent (envisioned as the most concentrated source of endocrine activity) on a monthly basis over the period of one year. Extractions using Oasis HLB were carried out on the same day of sampling, extracts were analysed (VM7Luc4E2 CALUX cells) within 4 days of sampling and results are expressed as biological equivalents (BEQs) relative to estradiol (ng E2-eq./L). By applying simple plug-flow/box models (3 boxes in total) between the 4 river sampling locations in water budget assessment, we were able to select 4 sampling events that meet our eligibility criteria (< 25% error in water fluxes due to uncertainty, spatial variation in rainfall, CSO events…). The BEQ loadings (g/day) calculated for these events indicate that the most upstream box can be considered closed, whereas the middle and most downstream box are either closed or show unaccounted loads that could be explained by point discharges of endocrine activity. Endocrine activities ranged from 0.7 to 16.5 ng E2-eq./L at individual locations within the Zenne River, comparable to WWTP effluent values. WWTP influent levels and hospital effluent ranged from 17.6 to 359ng E2-eq./L. In total, 9-57% of the BEQ contribution to the Zenne River downstream of Brussels was accounted for by the WWTPs. These results indicate that domestic (treated) wastewater is the largest contributor of BEQs in the Zenne River and would act conservative during transport in the river; furthermore, higher contributions are possible when CSO events occur whereby untreated portions of waste water are released into the river resulting in the highest river BEQs (16.5ng E2-eq./L) measured during this study.

MO050

Spatial and Temporal Distribution of Pharmaceuticals in the Humber Estuary, UK

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Pharmaceuticals are being increasingly detected at potentially harmful concentrations in the aquatic environment. However, monitoring programs often lack the detail required to determine spatial and/or temporal patterns. Such studies are limited in freshwaters and, even more so, in marine waters with no understanding of the interactions between these environments. The physical characteristics of an estuary as it moves from river to the sea, such as pH, abundance of fine particles, dilution potential and turbulence, have the potential to alter the concentrations and distribution of pharmaceutical compounds. In order to better assess the environmental risk of pharmaceuticals, more needs to be understood about the occurrence and fate of these compounds. This study will monitor the spatial and temporal distribution of six pharmaceuticals: acetaminophen, diclofenac, citalopram, metformin, ranitidine and trimethoprim, in the Humber Estuary. All of these compounds were deemed to be high risk based on their potential to enter the environment, persist and pose harm to aquatic organisms. The Humber Estuary is one of the largest estuaries in the UK and is fed by the Yorkshire Ouse, Trent and Hull rivers. Many parts of the Estuary are designated as special areas of conservation (SACs) due to the important habitats they provides for a wide range of species. Surface water and sediment samples will be collected from sites along a salinity gradient of the Estuary and sampling will be repeated bi-monthly for a year to determine spatial and temporal concentrations of the compounds. In order to provide wider context, surface water and sediment samples will also be collected from other estuaries within the UK. Preliminary data will be presented here and the project will provide a deeper understanding of the occurrence and fate of pharmaceuticals to better inform risk assessment, monitoring and identification of organisms at risk.

MO051

Estimating sewer residence time at the national scale to enable probabilistic risk assessment of down-the-drain household consumer product ingredients

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Many household consumer product ingredients disposed of down-the-drain can undergo significant degradation in the sewer system prior to being treated and discharged from a wastewater treatment facility. Understanding the distribution of sewer residence times for wastewater at the national scale, in combination with in-sewer biodegradation data for specific chemicals, can provide a more realistic assessment of environmental exposure and risk. However, the availability of data for sewer residence times at the national or regional scale is currently limited. We overview how commonly-available data resources such as road networks, land use and population data, and wastewater treatment facility data can be analyzed spatially to estimate the distribution of sewer residence times at a national or regional scale. This approach was developed using case study sewer system data and extrapolated to a national dataset of over 3,400 wastewater treatment facilities across the U.S., yielding a national median residence time of 3.3 hours. We demonstrate how sewer residence time distributions derived by this spatial approach can be used as a tool to enable probabilistic risk assessment of down-the-drain household consumer product ingredients for a given country or region.

MO052

Photolysis of Piperacillin - Kinetics, Transformation Products and the Meaning for the Antibacterial Activity

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Piperacillin - a bactericide β-lactam antibiotic - is one of the most essential drugs used in human and veterinary medicine to treat infections caused by Gram positive bacteria. Due to its high consumption, it can reach wastewater treatment plant in significant concentration and end up in the aquatic environment. Therefore the risk of the development and spread of resistant bacteria is a concern. While a variety of studies dealt with the degradation by microorganisms and hydrolysis of β-lactam antibiotics, the photolysis of these substances is not fully understood. This project focuses on the photolysis by UV-light (254 nm) and simulated sunlight. The previously developed method using online solid phase extraction coupled with LC-MS/MS allows analyzing piperacillin and other β-lactam antibiotics with limits of detection between 200 and 500 pg/L. In photolytic experiments piperacillin was irradiated until 80 % of the removal was reached. The photolysis using UV-light was compared with sunlight simulation in surface water. The first order half life time in the UV-light experiments (4.5 kW/m^2) was calculated as 17.4 min. In total, 10 transformation products (TPs) were detected. LC-QToF measurements were used for elucidation of their structures and monitoring of their formation over irradiation time. Mineralization measured as removal of dissolved organic carbon (DOC) was not observed. Further experiments aim to determine the antibacterial activity of these TPs to evaluate their risk concerning the development of resistant bacteria. Finally, surface water samples are planned to be analyzed to clarify if these TPs are also formed in the aquatic environment.

MO053

The role of wastewater irrigation on the introduction and fate of pharmaceuticals in soils

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Wastewater re-used for irrigation is currently not included in environmental risk assessments for active pharmaceutical ingredients (APIs) in soils. In lower and lower middle income countries, wastewater treatment plants (WWTP) may be of poor quality or not used at all, meaning there will be incomplete removal of APIs in the wastewater that is used for irrigation in arid areas. The addition of wastewater to soils changes the organic content and can increase the pH of soils, which will have an impact on the fate of any ionisable APIs introduced during the irrigation process. In addition, it is likely that the microbial community in the soil will be altered after addition of wastewater, which may lead to a changes to, and occurrence of, API biotransformation products. As the input of APIs to soil from wastewater irrigation is not currently included in the risk assessments, this is an area that requires increased attention. Terrestrial risk assessments are also not conducted for APIs which have an organic carbon partitioning coefficient (K_{-oc}) > 10 000; so not only is irrigation excluded we also lack risk assessment data for polar APIs. This study was undertaken using a modified sorption-desorption batch equilibrium method (OECD 106) to simulate the addition of wastewater to soils. The APIs studied were propranolol hydrochloride, naproxen and ofloxacin, and represent a range of API physico-chemical properties. They are widely used in lower and lower middle income countries. The wastewater used for this experiment was secondary wastewater from a WWTP in the UK and a synthetic primary wastewater with contrasting properties. The impact of wastewater additions to the soils on API fate and transformation was assessed in tandem with changes in pH, DOC concentrations and total enzyme activity (as a proxy for changes to the microbial community). Data from these experiments will provide insights into the impacts of wastewater irrigation on API fate in soils, and will contribute to improvements to the environmental risk assessments currently in place.

In situ measurement of nanoparticles (P)

MO054

Single Particle ICP-MS (SP-ICP-MS) For the Detection of Metal-Based Nanoparticles in Environmental Matrices

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During the last decade, the production and use of engineered nanomaterials (ENMs) have experienced a drastic increase, resulting in a potential risk of their release into the environment. Therefore, the study of their impact on the environment becomes crucial. The appropriate ecological risk assessment and management of ENMs in the environment requires quantitative measurements of both exposure and effects[1] that should, ideally, be performed by in situ analysis and give physicochemical characterization. However, most analytical techniques are not suitable for environmental matrices since nanoparticle concentrations are typically very low[2]. Historically, particle size has been measured by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM), while dissolved content has been measured by ultrafiltration followed by inductively coupled plasma mass spectrometry (ICP-MS) measurements. These common techniques have known limitations for measuring low concentrations in the presence of colloidal species in complex waters. Alternatively, single particle inductively coupled plasma mass spectrometry (SP-ICP-MS) has been found to be a promising technique for detecting and characterizing metal nanoparticles at very low concentrations. SP-ICP-MS is fast and efficient and can provide more information than other currently available techniques. It can lead to the determination of particle size, size distribution, particle number concentration, and the concentration of dissolved metal. Moreover, it can distinguish between particles of different elements. The aim of this work is to investigate the efficiency of SP-ICP-MS for the detection and characterization of metal nanoparticles in environmental waters where they can be involved in various physicochemical processes as shown by Figure 1

MO055

Multi-functional membranes for the removal of non-polar water pollutants and (bio)fouling prevention

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The nanotechnology revolution has a great potential to enhance a wide variety of products and services. In water treatment sector, particulary for the manufacturing of (re)active membranes, nanotechnology offers the opportunity for compact designs based on the properties available at the nanoscale and the ability to integrate multiple functionalities into a single product[1]. However, this promise can be offset by the concern of poor polymer biodegradability and the need for materials allowing a more efficient energy usage. Promising solutions include electrospun fibres based on environmentally friendly polymers such as poly(acrylic acid) (PAA) and polyvinyl alcohol (PVA)[2]. The modern nano-chemistry has demonstrated that dendrimers can be an effective solution, by combining their unique structural characteristics, chemical properties and highly tailored functionalities[3]. In this work, we prepared PAMAMG3-NH_2 dendrimer anchored PAA/PVA electrospun fibers as multi-functional membranes combining antimicrobial action with the capacity of retaining low-molecular weight non-polar pollutants from aqueous solutions. The antimicrobial action has been tested with strains of *Escherichia coli* and *Staphylococcus aureus* by studying their capacity to form new colonies and their metabolic impairment. The removal of aqueous pollutants was assayed using toluene at low concentrations. The new membranes were successfully tested for their ability to remove toluene from aqueous solution (?>98%) at the same time showing strong antimicrobial activity against the growth of the bacteria. **References** [1] Gilbertson L.M., Zimmerman J.B., Plata D.L., Hutchison J.E., Anastas P.T. 2015. Designing nanomaterials to maximize performance and minimize undesirable implications guided by the Principles of Green Chemistry. Chem. Soc. Rev., DOI: 10.1039/c4cs00445k [2] Santiago-Morales J., Amariei G., Letón P., Rosal R. 2016. Antimicrobial activity of poly(vinyl alcohol)-poly(acrylic acid) electrospun nanofibers, Colloids Surf., B. 146, 144-151. [3] Vunain E., Mishra A.K., Mamba B.B. 2016. Dendrimers, mesoporous silicas and chitosan-based nanosorbents for the removal of heavy-metal ions: A Review. Int. J. Biol. Macromol. 86, 570–586.
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MO056

Photocatalytic decomposition of diclofenac sodium in aqueous solution by nanopowder mixture ZnO/TiO2

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The xenobiotics, regarded as emerging contaminants, are present in trace quantities in aquatic matrix. Among a wide range of pharmaceuticals, synthetic, non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most frequently detected pharmaceutical residues according to their widespread availability. Diclofenac (2-(2,6-dichlorophenylamino)phenylacetic acid, DCF) is commonly used NSAID, resistant to biodegradation and about 15% of DCF is excreted unchanged after usage. The heterogeneous photocatalysis based on interaction of UV irradiation and semiconductor was studied in decomposition of diclofenac by nanoparticle mixture ZnO/TiO_2. ZnO/TiO_2 nanoparticles powder mixture was prepared by a simple solid-state method,where starting precursors were ground in an agate mortar in molar ratio 2:1, annealed at 700°C in air for two hours and ground again. Photocatalytic experiment was conducted in batch mode with initial concentration, 5 mg/l of diclofenac. The kinetic of diclofenac sodium photodegradation was monitored in different time intervals (range 5-60 minutes). The variations in diclofenac concentrations were analyzed by reversed phase HPLC (Agilent 1260) with diode array detector at 276 nm. After 30 minutes, removal efficiency was 97% and after one hour under UV exposure, complete degradation of diclofenac was achieved. According to obtained results, the photocatalytic decomposition by nanopowder mixture could be considered as a promising method for removal of diclofenac. Future research should be focused on optimization of photodegradation process, which includes: concentration of catalyst, time of irradiation, initial concentration of investigated pollutant, effect of pH value, water composition and identification of byproducts. **Acknowledgement:** The presented research is partly financed within a project of the Government of Vojvodina within Project No. 114-451-1821/2016-03.

MO057

Investigation of nanomaterial behaviour in the end-of-life phase of emerging photovoltaic technologies: The case of organic and perovskite cells

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Emerging photovoltaic technologies such as organic (OPV) or perovskite (PPV) cells are extremely thin-film solar cells, which have a maximum thickness of 400nm. Based on their thin-film technology, the cells are lightweight, flexible, and printable. To enable the deposition or printing of the different layer and the semiconductor, engineered nanomaterials (ENM) are applied. As to the end-of-life phase, up to now no specific recycling technologies can be expected. Therefore, dependent from the respective national waste management system, it can be expected that these material will either be incinerated or land-filled. This is why specifically the risk of possible land-filling has to be taken into account in LCA. However, up to now no empirical data on the behaviour of ENM in a landfill are available. In addition, also in the use phase a possible emission could be envisaged in case of damaged cells. Also here, no empirical insights are available. In order to provide first estimates on possible leaching of ENM, we conducted laboratory leaching experiments for the two emerging photovoltaics: OPV and PPV. For our experiments, we considered damaged OPV and PPV cells in the following three future applications during the use phase and at the end-of-life in a landfill: A) organic solar module, B) perovskites as an extra layer on silicon modules, and C) OPV and PPV as a portable mobile charger. The leaching of the use phase was done with distilled water as leaching solutions in accordance to the standard DIN EN 12457-4; and the leaching of the landfilled cells were conducted according to the aqua regia extraction method of the DIN EN 13346. The elemental analysis is performed with the inductively-coupled-plasma optical-emission-spectroscopy (ICP-OES). The first results of the leaching with aqua regia show that almost 100% of the included metals of OPV and PPV were leached out. The leached metals of the PPV were lead, lithium, and gold from the electrode; the OPV samples emitted silver, aluminium, and zinc. The results are pending further analysis as to what fraction of the dissolved metals and substances were actually "nano"-particles versus ionic species or otherwise. Based on prior studies, it is anticipated that ENM may leach at fairly low percentages of the total leached amount. All in all, the current and coming results give empirical insights of the emissions of future OPV and PPV applications in landfills.

MO058

Degradation of solid state C60 fullerene by UV irradiation under environmentally relevant conditions

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Carbon-based nanomaterials, such as C_{60} fullerenes, are expected to accumulate in soils and sediments. Although little is known about their environmental fate, these nanoparticles may be susceptible to photochemical and microbial degradation. In the present work, C60 spiked onto a glass surface or added to silicon dioxide or sandy soil was exposed to UV light. Upon UV exposure, the fullerenes were degraded in all the treatments and the decay followed a pseudo-first-order rate law. In absence of a matrix, the half-life ($t_{1/2}$) of the C_{60} was 13.1 days, with an overall degradation of 45.1% that was accompanied by the formation of functionalized C_{60}-like structures. Furthermore, mass spectrometric analysis highlighted the presence of a large number of fulleroid products that were not directly related to the irradiation and showed opened cage structures and various degrees of oxidation. When C60 was spiked onto solid matrices the degradation occurred at a faster rate ($t_{1/2}$ of 4.5 and 0.8 days for silicon dioxide and sandy soil, respectively) and minor but consisted losses were found in the non-irradiated samples, presumably due to biotic processes and/or a matrix effect in these samples. The results of this study suggest that light-mediated transformation of the fullerenes will occur in the environment and that an accurate assessment of their fate is complicated by the large number of products that may be formed.

MO059

Avoidance behaviour of Enchytraeus albidus (Oligochaeta) after exposure to AgNPs and AgNO3 at fluctuating temperatures

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To assess the effects of fluctuating temperature on the avoidance behavior of *Enchytraeus albidus* towards silver nanoparticles (AgNPs) and silver nitrate ($AgNO_3$), 48h avoidance experiments were carried out at 20?C and at temperature fluctuating daily between 15 and 25?C. *E. albidus* was offered to choose between clean and spiked (10, 20 and 40 mg Ag/Kg dry soil) OECD artificial soil. At the end of the experiments, the worms found in the clean soil (those who avoided the selected toxicants) were processed for silver tissue contents in order to find out whether these worms had explored the contaminated soil prior to avoiding it. The results revealed that at 20?C, *E. albidus* avoided both AgNPs and $AgNO_3$ and that there was no difference in avoidance towards either chemical. At fluctuating temperature, *E. albidus* failed to avoid AgNPs at the lowest concentration (10 mg Ag/Kg). For the other treatments, there was no difference in avoidance towards either chemical. Ag^+ tissue contents revealed that at 20?C, in the lowest treatment (10 mg Ag/Kg) *E. albidus* accumulated significantly more Ag^+ from $AgNO_3$ than AgNPs. For the other treatments, there was no difference in Ag^+ accumulation. Ag tissue contents at fluctuating temperature were similar between the worms exposed to the homologous treatments of AgNPs and $AgNO_3$. Our findings reveal that natural daily temperature fluctuation may impact avoidance behaviour towards metals and metal nanoparticles differently than observed under laboratory conditions where tests are typically conducted at constant temperature. Evidence of Ag^+ tissue accumulation in the worms found in clean soil indicates that *E. albidus* interacts with the contaminated soil prior to deciding to avoid it.

MO060

Eco-friendly profile of pegylated nanographene oxide synthesized for medical application

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Nanographene oxide (nGO) was recently explored into the biomedical field for the development of new antitumoral therapeutic agents. This new multifunctional nanoplatform shows interesting potential as a contrast agent for bioimaging and on cancer therapy by hyperthermia. The therapeutic mechanism is based on energy transfer due to nGOs' absorbance in the near infrared region (650 - 950 nm), usually known as first optical therapeutic window due to the high depth penetration on tissues, causing tumor cell death by hyperthermia above 43ºC. After treatment organisms will inevitably excrete the nanosystems, which will end up being released into the environment. Thus, following the life-cycle of materials requested by several international legislations, it is essential to understand the potential impacts of nGO to the environment. To evaluate the hazard of pegylated nanographene oxide (nGO-PEG), three key species from three different trophic levels were used: the green micro-algae *Raphidocelis subcapitata* (growth inhibition test), the cladocera *Daphnia magna* (acute and chronic tests), and the fish *Danio rerio* (FET test). To asses if the nGO-PEG ingested by the daphnids had any influence on survival by heating through solar irradiation, since nGO-PEG absorbs in the near-infrared window, neonate and adult daphnids were exposed outdoors from sunrise to sunset on a sunny clear day. A light wave length exposure was also carried out under an infrared lamp to evaluate the same effect (influence of heating on survival) with *Daphnia magna* and *Danio rerio*. Additionally, and by taking advantage of the phenotypic transparency of *D. magna*, nGO-PEG was fluorescently tagged in order to evaluate the potential entrance of nGO-PEG. *R. subcapitata* growth inhibition test showed effects during the first 48h, recovering till the end of the test (96h). No acute or chronic effects were observed for *D. magna,* although confocal microscope images showed nGO-PEG uptake. The solar exposure with *D. magna* caused mortality due to the elevated temperatures during the exposure. No mortality was observed in the infrared exposure. As for the FET test, very small percentages of mortality and abnormalities were observed in both exposures (with and without the infrared lamp). Concluding, nGO-PEG presented low to no hazard to all three key species used and therefore represents potentially no risk for the environment. Keywords: nanographene oxide, *Raphidocelis subcapitata*, *Daphnia magna*, *Danio rerio*

MO061

Environmental corona of metal oxide nanoparticles

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The behaviour of metal oxide nanoparticles, particularly copper oxide (CuO) and cerium dioxide (CeO_2), is studied in a methodical approach in presence of biological and environmental compounds at variable concentrations. Nanoparticle agglomeration and stability are examined using dynamic and electrophoretic light scattering experiments enabling the measurement of hydrodynamic particle diameter and zeta potential values. The influence of small model substances such as benzoic acid up to larger molecules of natural organic matter (NOM) is investigated. Depending on the type of adsorbent destabilising and stabilising effects on nanoparticle suspensions were observed. In addition to the characterisation the sorption behaviour of environmental relevant substances on nanoparticle surfaces are evaluated and quantified in terms of sorption isotherms and isothermal titration calorimetry (ITC). Complementing these measurements, the fraction of dissolved copper ions and their interaction with biomolecules will be determined to correct the thermodynamic data of sorption isotherms and ITC experiments. The adsorption depends mainly on nanoparticle parameters like the specific surface area and is affected by electrostatic attractive and repulsive interactions as well as steric and van der Waals forces. Further work aims to elucidate if adsorbed biomolecules onto nanoparticle surfaces are still accessible for microbial degradation. Regarding the possible dissolution of CuO nanoparticles the copper ion toxicity towards bacteria from wastewater treatment plant sludge was tested. Especially for metal and metal oxide nanoparticles the dissolution processes related to corona formation are relevant for the fate and transformation of nanoparticles in the environment.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment (P)

MO062

Comparative analysis of the electrochemical behaviour of PGMs on modified SPCE and GCE surfaces for environmental matrices

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The increasing concentration of platinum group metals (PGMs) in the environment, are raising concerns about the environmental impact and toxicity of these elements in living organisms. The composite of reduced graphene oxide antimony nanoparticles (rGO-SbNPs) were drop-coated on a screen-printed carbon electrode and a glassy carbon electrode surfaces, respectively followed by voltammetric comparison of results. The present work is an experimental investigation dealing with the selection of the electrodes for the best detection of PGMs in environmental matrices. The electrochemical features of such electrodes have been fully evaluated with cyclic voltammetric and adsorptive differential pulse cathodic stripping voltammetric experiments. The results obtained using both electrodes exhibited similar electrochemical properties. The peak current observed in the modified electrodes is dependent on the porosity, nature and number of sites involved in partitioning the complex into the film. Compared to screen-printed carbon based electrode, which has relatively small surface area, the glassy carbon electrode demonstrated almost the same application performances. The results therefore exhibited strong, stable and reliable cyclic and strpping voltammetric peak currents. The rGO- SbNPs/SPCE sensor exhibited a detection limit of 0.45, 0.49 and 0.49 pg/L for Pd(II), Pt(II) and Rh(III) respectively. The rGO- SbNPs/GCE sensor exhibited a detection limit of 0.42, 0.26 and 0.34 pg/L for Pd(II), Pt(II) and Rh(III) respectively. The differences and similarities in the results obtained are discussed in this paper.

MO063

Concentrations of butyltins in Scottish marine sediments: improved detection using Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS)

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Although it is 14 years since the global ban on the application of tributyltin (TBT)-based antifoulants to ships, and 9 years since the complete ban its presence on vessels, TBT and its metabolites continue to be significant environmental hazards. Whilst recent studies on imposex in marine gastropods show that the degree of exposure to butyltins is decreasing, imposex due to organotins exposure is still apparent in some areas; furthermore, organotins continue to be used as plastic stabilisers and as wood preservatives. As TBT degrades only slowly in anoxic sediments, fine grained sediments within harbours and in the vicinity of shipyards/dry docks remain a significant reservoir of contamination. Frequently, disturbance of these sediments is unavoidable in order to maintain navigable access. Dredging and the disposal of spoil is therefore a potentially significant source of butyltins to the wider marine environment and the OSPAR convention requires monitoring of the amount of butyltins disposed of to sea via dredged spoil. Additionally, post-disposal monitoring is required to ensure that contaminant concentrations at dredged spoil disposal sites do not pose a hazard to the marine environment. Finally, TBT is a Priority Substance that requires monitoring for the WFD and under the OSPAR Coordinated Environment Monitoring Programme. MSS has recently improved analytical method performance by introducing the use of ID-GCMS quantification. The method uses shaken methanolic acetic acid extraction, sodium tetraethylborate derivatisation of the extracted organotins and simultaneous extraction of the ethylated butyltins into *iso*-hexane, deactivated silica clean-up before ID-GCMS. Performance was validated (LoQ, accuracy, uncertainty) to meet the QA/QC Directive (90/2009/EC) by the use of SOPH-1, BCR-646 and PACS-2 Certified Reference Materials, blank sediments and the QUASIMEME laboratory proficiency scheme. Recoveries are quantitative for DBT (ca. 85%) and TBT (ca. 95%), but not for MBT (ca. 50%), which is not extracted efficiently; the method detection limit is below the Swedish EQS for TBT (1.6 μg / kg d.wt.) that has been proposed to be used by OSPAR for assessing the status of marine sediments. Concentrations of butyltins in Scottish marine sediments are reported.

MO064

Transformation / Dissolution of Titanium Dioxide Grades in Environmental Media

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The assessment of the ecotoxicological potential of titanium dioxide grades is progressing through the REACH evaluation process. Conventionally, the aquatic hazard classification schemes for metal substances are primarily determined by the hazards elicited by their dissolved metal ions, and typically do not take exposures to metal substances that are not dissolved in the water column into account. Therefore, the transformation and dissolution of 6 different nano- and micro-TiO_2 grades were investigated according to OECD Monograph No. 29: Guidance Document on Transformation / Dissolution (TD) of Metals and Metal Compounds in Aqueous Media (2001 and under Good Laboratory Practice. The T/D protocol, including conditions and media representative of those generally occurring in the aqueous environment, was applied to determine the rate and extent to which TiO_2 grades in solution remain dispersed or produce ionic Ti species. The six different commercial TiO_2 grades tested varied in size, specific surface area and crystalline structure. Six T/D studies were performed with a loading of 1 mg TiO_2/L of each grade at pH of 6 and 8 for 28 days, respectively. Solutions were sampled for the analysis of Ti concentrations after 1, 7, 14 and 28 days. Samples were filtered through polyethersulfone membranes with a pore size of 200 nm and 3-kDa membranes via centrifugal filtration (corresponding to a pore size of approximately 2 nm) to separate the operationally-defined dispersed and dissolved fractions. The feasibility of the method was validated with a commercial standard containing a certified dissolved Ti concentration; titanium in the respective filtrate was fully recovered (100 ± 15 %). ICP-OES was used to quantify aqueous Ti concentrations since calcium contained in the standard T/D media may interfere with ICP-MS measurements. Titanium concentrations of all solution samples after centrifugal filtration were below the limit of quantification or detection (< 300 ng/L). Dispersed Ti concentrations up to 5 μg/L were measured for some materials at pH 6 whereas dispersed Ti concentrations of solutions at pH 8 were below the detection limit. The results indicate that all 6 different commercial nano- and micro-TiO_2 grades do not release detectable soluble ionic Ti species in aqueous media under standard T/D conditions.

MO065

The Development of an Efficient Methodology for the Matrix Separation of Environmental Nickel Samples in the Norwegian Arctic for Nickel Isotope Analysis

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A challenge associated with nickel (Ni) pollution is source tracing of Ni and separation from other metals and organic matter for the purpose of isotope analysis. To identify Ni sources in the environment, we collected snow, soil, lichen, and moss samples to process and determine its presence. We present the separation efficiency of Ni from the samples collected in the Sør-Varanger area of Norway. The method of separation was adapted from Gueguen *et al.*[1] to accommodate the unique challenges (presence of organic matter and metals) associated with the samples. Before separation began, sample aliquots were evaporated to near dryness and mixed with H_2O_2 and HNO_3 to produce an oxidizing acidic mixture to aid in the dissolution of organic matter complexes in the samples. Once dissolved, 6 M HCl was added to the aliquots and they were ready for the first stage of separation. The first stage of separation employs an anionic resin, AG 1-X8, generated with 6 M HCl, which the sample was passed through and collected. Collected samples were evaporated to near dryness and treated again with the H_2O_2 and HNO_3 to remove the remaining organic matter complexes associated with Ni. Samples were then basicified with ammonium citrate (pH 8-9) and were ready for the second stage of separation. We used a Ni specific resin; Ni-DMG (pH 8-9, generated with ammonium citrate) and the samples were loaded onto the resin. Passing 3 M HNO3 through the column then broke up the Ni-DMG complex. Fractions were collected and evaporated to near dryness for analysis. Through the process of a two-stage ion exchange column chromatography separation and by using H_2O_2 and HNO_3, we were able to optimize purification. Removal of organic matter occurred successfully via the oxidizing acidic mixture, which is essential for analysis by thermal ionization mass spectrometry (TIMS). TIMS has lower ionization efficiency so is, therefore, extremely sensitive to any organic matter present. The presence of any organic matter could potentially preclude the ionization of the Ni in our analyte. The two-stage separation and use of analytically pure chemicals (ammonium citrate, H_2O_2 and HNO_3) were essential for removal of other metals/organics and purification of Ni. On average, the separation and purification of Ni produced a 91.9% success rate, demonstrating our method to be efficient and useful for further work in analysis of Ni in the environment. 1. B. Gueguen, *et al. Geostand. Geoanal. Res*. 37. 297. 2013. \n

MO066

Influence of the lead anions to plant growth in artificial soil condition

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A literature review on the role of anions of heavy metal salts in modifying the deleterious effects of pollutants reveals imprecise conclusions. Some authors indicate a clear relationship between toxicity and lead salts composed of different anions, the indication from others is one of inaccurate distinctions. To minimize the influence of external factors for the assessment of effects of heavy metals (particularly concerning plants) can be controlled in the laboratory experiments using standard artificial soil or soil extract. The purpose of this work was to compare the effect of different lead salts on the growth characteristics of plants using Phytotoxkit. The aim was try and quantify the median effective concentration of lead cations (Pb^{2+}) in reference to specific salts tested - nitrate, chloride or acetate. Toxicity of lead salts was investigated on germination and development of plant seeds of monocots *Avena sativa* and dicots Raphanus *sativus*. Toxicity was assessed using substrate and eluate methods employing Micrbiotest Phytotoxkit disposable plates. The substrate method entailed direct contact of seeds with the soil, whereas in the eluate method seeds were exposed to water extract of soil samples treated to varying degrees of lead salts. The soil used as artificial soil was prepared in accordance with international standard ISO 11268-2012. Lead salts tested were in the range 50-2500 mg/kg. Plant responses observed showed that: at lower concentrations up to 300 mg / kg growth of the vegetative part of the plant was enhanced whereas the root system was suppressed at these levels. Seedlings of *Avena sativa* exhibited significantly less inhibition to the toxicant concentration range tested than the seedlings of *Raphanus sativus*. The greatest inhibition of root growth was evident in the treatment using lead acetate (this was attributed to decrease in pH to 3-4) - at 1300 mg / kg. At this maximum inhibition with lead nitrate, in the case of soil extract (eluate method) inhibition was only 60%; and lead chloride - 30%.Thus, in the study of the toxicity of lead salts both with the eluate and substrate methods it was shown that the degree of growth inhibition of plant seedlings grown from seed depends on the nature of the anion. In particular, it was evident that lead nitrate was less toxic than the lead acetate.

MO067

Metal Distribution and Bioaccessibility in Urban Garden Soils in Victoria, BC, Canada

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Victoria City Council recently passed a number of bylaw changes allowing urban gardeners to grow and sell produce. Natural weathering, industrial activities, automobile exhausts, paints, and inorganic wood preservatives and herbicides can potentially introduce metals such as As, Cd, Cr, Cu, Ni, Pb and Zn into urban environments including garden soils. A baseline study was therefore conducted in collaboration with the Victoria Compost Education Centre (VCEE) to assess the distribution of metals in residential and community gardens in Victoria. The sampling program was initiated with a call for volunteer participants through advertisements in local newspapers and the VCEE website. Soil samples (137) were collected from respondents comprising 68 homes and community gardens in the municipalities of Victoria and Esquimalt. All the samples were analyzed for total metals using a portable X-ray fluorescence analyzer (XRF). A subset of the samples (30) was also analyzed by ICP-MS. There was strong correlation between the XRF and the ICP-MS data for a majority of the metals. An interactive map of the total metal distribution was developed and will be available to the public through the VCEE website. As, Cr, Cu, Ni, Pb and Zn concentrations in some of the samples exceeded Canadian environment soil quality guidelines for residential land use. Metal bioaccessibility, which was determined in a subset of the samples, was variable increasing in the order Cr ($6.8 \pm 2.6\%$) < Ni ($19 \pm 7\%$) < As ($27 \pm 7\%$) < Cu ($36 \pm 10\%$) < Zn ($63 \pm 13\%$) < Pb ($82 \pm 12\%$) < Cd ($86 \pm 16\%$). The relatively high Cd, Pb and Zn bioaccessibility suggested the risk associated with ingestion of metals in soils adhering to produce may be elevated at sites with elevated Cd, Pb and Zn concentrations. There was also the potential for plant uptake of these metals. However, the overall hazard indices and carcinogenic risks, incorporating bioaccessibility data, indicated that the general risk associated with ingestion of the metal contaminants is low except for the isolated hotspots. Fact sheets on "Soil Contamination" and "Best Practices for Healthy Urban Gardens" including recommendations for reducing exposure to metals and other contaminants in gardens have been developed by the VCEE and posted online. Ongoing work includes additional sampling and analysis of soils and vegetables in gardens with elevated metal levels and expansion of the program to other municipalities in the Greater Victoria area.

MO068

Metal concentrations and chemical partitioning in topsoil and road dust affected by different industrial activities

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Heavy metals are transferred to soils and environment from natural and anthropogenic sources. The fast industrialization in recent decades has supposed an increase of metalsemissions becoming a serious health problem in many countries. Soil and road dust can act both as source and sink of metals in the environment. Hence, the main aim of this study was to determine the influence of different industrial activities(service, refinery, fertilizer, power and tannery industries) in metal concentration and behaviour in soil and road dust samples. Results from industrial areas were compared with a natural area used as background. Physicochemical properties, metal content (Cd,Co,Cr,Cu,Ni,Pb,Zn) and chemical distribution of metals were determined. Thehighest accumulation of heavy metals was found in road dusts samples from the industrial areas,being Zn, Pb, Cr and Cu the metals with higher concentrations. Each industrial activity contributed differently to the concentration of metals in soil and dust. The highest concentrationsof Cr were found in areas affected bytannery industry, while Pb and Zn showed the highest values in that affected byrefinery and fertilizer industry. The chemical partitioning suggests that Cu and Cr are more easily mobilized from road dust than soil,while Ni and Co distribution is not affected by the industrial activity. In contrast, the industrial activity influences the physicochemical properties andbioavailability of metals insoils and road dust.

MO069

Plant growth and changes in redox potential modulate the effects of biochar for improving wetland soils polluted by mine wastes

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Mine activities affect territories far away from the mine site, when tailings are eroded and wastes transported and deposited in lowland areas such as wetlands. This study aimed to evaluate the combined effects of biochar addition (from pruning trees -BPT- and from sewage sludge -BSS-) and plant growth (*Sarcocornia fruticosa*, *Sf*) for the phytomanagement of wetland soils affected by mine wastes. A greenhouse experiment was performed with mine wastes (MW) of pH~7.4 collected from the mining district of Cartagena-La Unión (SE Spain). Six treatments were assayed: MW; MW+Sf; MW+BPT; MW+BPT+Sf; MW+BSS; MW+BSS+Sf. PVC columns (15 x 30 cm) were filled with the corresponding soil treatment and seedlings of *Sf* were planted. The columns were put inside bigger containers in which changes in the water table level were simulated. For the first month the containers were filled with tap water (water level ~5 cm above the column top, high water level phase -HWL-), while for the second month half of the water was removed (water level ~15 cm below the column top, low water level phase -LWL-). This alternating cycle of HWL-LWL phases was repeated four times. The pH and redox potential (Eh) were regularly checked and porewater samples extrated for measuring water soluble organic carbon (WSOC) and metal concentrations (Cd, Fe, Mn, Pb and Zn). The addition of biochar and the presence of *Sf* did not affect the pH. Both types of biochar favoured decreasing Eh values, due to the microbial activity stimulation by the WSOC input. This effect was more intense with biochar BSS due to the greater WSOC content. Fe, Mn and Zn porewater concentrations were affected by the treatments assayed, showing higher concentrations those with biochar addition, probably due to the metal oxides solubilisation at lower Eh values. The presence of *Sf* contributed to mobilise these three metals towards the upper part of the columns, due to the capillary upward movement of water and solutes. On the contrary, Cd and Pb porewater concentrations were not affected neither by the addition of biochar nor by the presence of *Sf*; all the treatments assayed showed similar concentrations. A tendency to decrease Cd and Pb porewater concentrations during HWL phases and to increase during LWL phases was shown. The effectiveness of biochar addtion differed for different metals and was influenced by changes in the water table level and the presence of *Sarcocornia fruticosa*.

MO070

Derivation of soil threshold concentrations for arsenic, copper, and nickel: Results of chemical extraction methods

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In the German Federal Soil Protection Act, soil precautionary values are defined as "soil values which, if exceeded, shall normally mean there is reason that concern for a harmful soil change exists". The current soil precautionary values were derived from total contents (aqua regia extraction) of metals. Regarding the pathway "soil – soil organisms", there is a poor correlation between total metal contents and their effects on microbes, plants and invertebrates. A realistic risk assessment of metals should preferentially consider bioavailibility of metals in soil, but it is not clear which fraction should be used for regulatory purposes and how to measure it. Therefore, a project was established that aims to correlate results of several chemical extraction methods with ecotoxicity data of chronic effects on different organisms in order to identify the most promising measure for the bioavailable metal fraction in soils. One part of the project was dedicated to appropriate extraction methods and first results will be presented. Soils selected for the study cover a wide range of Central European soil properties (e.g. 0.9-23.3 % organic carbon; 5-36 % clay). Replicates of these eleven soils were spiked with the cationic metals copper and nickel and the oxyanion arsenate. Then, next to the aqua regia extraction, metals will also be extracted by different neutral salts ($Ca(NO3)_2$ adjusted to soil ionic strength, 0.01 M $CaCl_2$, and 1 M NH_4NO_3), chelating agent ($DTPA/CaCl_2$), or weak acid (043 M HNO_3). The extraction strength of the different methods follows the order HNO_3 > DTPA > NH_4NO_3 > $CaCl_2$ for most soils. However, the amount of extracted metal depends on the type of soil. The influence of soil properties on the amount of extracted metals will be further evaluated. Those informations will be combined with the ecotoxicity data and later on, it will be used to propose a new concept for including bioavailability into the derivation of future precautionary values.

MO071

Bioaccessibility, mobility, and health risk assessment of cadmium in farmland pollution soil in Taiwan

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Wastewater containing cadmium (Cd) is frequently used for irrigation in Taiwan, therefore Cd could be promptly transferred into soils and plants. Cd is known as the metal with high toxicity that can directly and indirectly induce the production of reactive oxygen species (ROS) that might damage cell macromolecules, especially polyunsaturated lipids and proteins. With long-term irragulation containing Cd, Cd would accumulate in soil and then become accessible to plants and human, furthur causing health injury; therefore this issue is highly needed being noticed. According to the Taiwan EPA statistical data, Taoyuan City in Northern Taiwan has apparently serious domestic farmland pollution issues with Cd. Besides, there are still much potential pollution in the agricultural land without being regulated. In this study, two *in-vitro* assays, physiologically based extraction test (PBET) and simplified bio-accessibility extraction (SBET) were used to discuss the bioaccessibility of Cd in contaminated farmland in Taoyuan City. The solubility and mobility of Cd in soil were evaluated through a sequential extraction procedure (SEP) to identify the relationship between the chemical forms of Cd and its bioavailability in soil, which could be further used as the reference of exposure dose in health risk assessment for farmers and sensitive populations such as children. In this study, the total Cd content in the studied farmland soil was between 6.95 and 33.03 mg/kg, the bioavailability based on SBET was between 64.6 and 96.3%. Results from the two-phase extraction showed that the propotion of Cd in gastric extraction (62.50–98.29%) was larger than the small intestine one (12.91–45.97%). To understand the effects of soil parameters on the bioaccessibility of Cd, Pearson analysis was conducted with all the 18 soils. Based on PBET tests, it could observe that concentration of Cd in gastric extration was positively correlated with sand and organic carbon contents in soils; namely the higher contents of sand and organic carbon in soils resulted in greater Cd extraction. In conclusion, the proporties of soils, such as organic carbon, pH, and particle size also had significant correlation with bioaccessibility of Cd. According to the aforementioned results, a health risk assessment for farmers and sensitive populations can be conducted. The obtained results provide suggestions to decision makers on setting new strategies in the risk management of Cd-contaminated soil sites.

MO072

Evolution of residual polluted soils after ecological restoration

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In 1998, the holding pond of a pyrite mine in Aznalcóllar (SW Spain) broke open, spilling around 6 x 10^6 m^3 of acidic waters and tailings with high concentrations in heavy metals and related elements, affecting 4600 ha of agricultural soils. In the following years, the restoration activities involved the removal of tailings and heavily polluted soils, the extensive application of organic and inorganic amendments, and the general phytostabilization of the area; finally the recovered area was established as the Guadiamar Green Corridor. Nowadays, 18 years after the accident, patches of soils affected with residual pollution are still found in the area. These patches are easily identified in field by the total absence of vegetation surrounded by gradually changing plant communities. We studied soil physicochemical properties and constituents and we assessed the mobility and bioavailability of arsenic and heavy metals (Cu, Zn, Cd and Pb) by the use of selective extractions. Our results indicated that the main factors limiting the plant growth in the areas without vegetation are the high acidity, the low content in organic matter and the low cation exchange capacity. High total concentrations of As and Pb were detected in the unvegetated areas, but high values in soluble and bioavailable Cu, Zn and Cd were observed in the surrounding vegetated areas, indicating a potential risk of dispersion of the pollution to the environment. We also assessed the bioaccumulation of trace elements in plants growing in the areas close to the unvegetated soils. Our results indicated that trace element bioaccumulation is strongly related to the differences observed in the richness and abundance of plant species. We found that the specie *Lamarckia aurea* L. was dominant in heavily contaminated soils and *Trifolium repens* L. was the best adapted to the moderate levels of pollution. We observed high levels of accumulation of trace elements in the case of As and Pb, with BAF values higher in the moderately contaminated areas than in the recovered soils; although the accumulation of these elements were mainly in the root system of plants. The concentrations found in the aerial parts of the plants indicated a potential risk of dispersion of the pollution through the food chain in the case of Cu and Cd, which should be monitored over time.

MO073

Lanthanide distribution across environmental compartments in a LREE enriched geological area in Quebec (Canada)

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Lanthanide (LN) use in industry, medicine and agriculture has increased during the last decades, leading to a dramatic rise in their demand. Understanding the distribution of LN in areas naturally enriched is thus necessary to predict possible risks associated with their transfer in prospective mining areas. This “ground zero” state of play is key to monitor mining activity contribution in a potential LN enrichment in surrounding environments during and after mining activities, and their subsequent rehabilitation. For this study, a LREE enriched geological area has been targeted (Montviel prospect, Quebec). Three aquatic sites were selected above different geological material: pyroxenite (A) ferrocarbonatite (B) and polygenic breccia (C). Parent material (PM), sediment (SED), river water (RW) and suspended material (SM) were sampled at each point. Sediment porewaters (PW) were obtained by centrifuging, and *Thypa latifolia* L. (TY) was collected as a representative plant. LN concentrations were determined by ICP-MS. Table 1 shows the main properties of sediments and total LN concentrations (TLN) of the 3 studied sites. Site B showed the greatest concentration in PM, but TLN in SED, PW, RW and SM had the lowest values; this can be attributed to the difference in sediment properties (B= lowest content in Fe, Mn and clay and really high TOC content). In the water fraction, site A showed the highest TLN in RW, but concentration in SM did not follow a relation with RW. To better understand availability, partitioning coefficients (Kd) for LREE were calculated. Results for SED/PW-Kd were B>A>C (in site B, LREE are preferentially retained by sediments); for SM/RW-Kd were C>A>B (in site B LREE mostly remain in waters). **Table 1.** Main properties of sediments and TLN in PM, SED, RW, PW and SM. Bioaccumulation factors (BAF) with SED and bioconcentration factors (BCF) with PW and RW were calculated for TY roots, stems and leaves. The most interesting result appeared in A, where root-BAF was close to 1, suggesting LN accumulation; and great BCF appeared in root and stem which implies better phytoaccumulation. Sites B and C showed opposite results, thus, LN phytoaccumulation capability could result from sediment-water composition and not only from TLN.

MO074

Bioavailability of Cu, Cd and Zn toward Chlorella vulgaris in two Siberian rivers

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It is well known that an adverse effect of heavy metals (HM) on aquatic species depends on water chemistry. One of the main components that can influence metal toxicity in waterbodies is humic substances (HS). Assessment of metals hazard for rivers can be complicated as HS levels in rivers can significantly vary. The aim of this study is to compare the bioavailability of Cu, Cd and Zn to microalga Chlorella vulgaris in two Siberian rivers. Water samples were collected from the Enisey river and its tributary Kacha river. These samples were spiked with HM before testing. Algal growth and photosynthetic activity inhibition tests were conducted to estimate the toxicity of heavy metals to Chlorella vulgaris thermophile strain culture. The unique equipment and methodology were employed for acute and rapid assay performance. The toxic effect of heavy metals was observed to decrease compared to the control in the water samples obtained from both rivers. The average toxicity of Cu2+ was a factor 8 and 87 lower in the water samples from the Enisey and Kacha rivers respectively. Thus, bioavailability of copper in a larger Enisey river was almost 11 times higher. The toxicity decrease of Cd and Zn was less revealed in both waterbodies. As the key role of HS in metal complexation has been widely discussed, we estimated the toxic effects of the three heavy metals on Chlorella vulgaris in the presence of humic substances. The results showed that toxicity decrease caused by the presence of humic and fulvic acids was the lowest for Zn2+ and the highest for Cu2+. Thus, copper toxicity was the most reduced both in natural waters and in the media containing HS among the three assessed metals. In addition, the bioavailability of the heavy metals in natural waters varied through the year being higher in spring and summer. The results show a higher ability of the Kacha river to reduce heavy metal toxicity compared with the Enisey river. This difference may be caused by a high level of humic substances in the Kacha river as well as anthropogenic organic compounds that can also contribute to the binding capacity of this small tributary.

MO075

Metal complexation in sewage effluent

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The shift toward bioavailability-based standards for metals such as copper and zinc improves the ecological relevance of the standard but also introduces significant complexity into assessing compliance since this requires consideration for the influence of physico-chemical characteristics such as pH, DOC and hardness. This study examined differences in the copper and zinc complexation characteristics of effluents from a range of different sewage treatment works, and in relation to so-called ‘natural’ samples. This information is essential to determine whether the inclusion of effluent-specific complexation characteristics within the regulatory framework could further enhance the environmental relevance of compliance criteria. The study findings show that the differences in copper complexation characteristics between effluents from different sewage treatment works were of a similar magnitude to the differences between that of effluents and samples from natural sources. Zinc complexation characteristics of the effluents were substantially different from that of natural samples. The capacity of the effluents to complex zinc vastly exceeded that available in natural samples indicating the presence of a category of anthropogenic ligand with a high affinity for zinc. The data also show that the copper and zinc complexation characteristics of effluent samples obtained from the same treatment works were less different from one another than to effluents from other treatment works and therefore that sewage source can have an important influence on complexation characteristics.

MO076

Predicting copper and zinc speciation in estuarine waters

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A new generation of speciation-based aquatic environmental quality standards (EQS) for metals takes bioavailability into account. Regulators are increasingly using models such as the biotic ligand model (BLM) to predict the free metal ion concentration and its ambient toxicity to set site-specific EQS. Some countries such as the UK have set a new marine EQS for copper and zinc, with the copper standard based on an empirical relationship between copper toxicity to mussels (*Mytilus* sp.) and ambient dissolved organic carbon (DOC) concentrations. At low DOC concentrations the new EQS is more stringent, but above 162 μM DOC it is higher than the previous value. However, the relationship between DOC and zinc and copper speciation is poorly defined in estuarine waters. This hampers the development and application of a BLM for these vulnerable habitats that are often exposed to elevated metal concentrations. This research compared measured estuarine metal speciation data with that derived from available models including WHAM VII and Visual Minteq. It was concluded that DOC is not necessarily the best surrogate for metal complexation in estuaries. Nevertheless, the determination of ligand strength and concentrations by Competitive Ligand Exchange Adsorptive Cathodic Stripping Voltammetry enabled the prediction of the most ecotoxicologically relevant metal species, the free metal ion, within an order of magnitude for estuarine waters by using readily available metal speciation models.

MO077

A pH-controlled flow-through system exposing fathead minnows and rainbow trout to copper under soft water conditions with added dissolved organic carbon

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A series of toxicity tests were performed to evaluate the chronic toxicity of copper (Cu) to fathead minnows and rainbow trout under various pH conditions (pH 6 - 7). Potential effects on Cu bioavailability and toxicity attributable to water physicochemical characteristics were evaluated. Fish were exposed to Cu concentrations in low hardness water (~40 mg/L as $CaCO_3$) with added dissolved organic carbon (DOC; 1 – 1.5 mg/L), at pHs ranging from 6 to 7. All tests were conducted under flow-through conditions. Cu exposure solutions (as $CuSO_4$ x $5H_2O$) were equilibrated for a minimum of 2 hours after preparation and prior to fish exposure after mixing with dilution water at the target pH. Prior to test initiation, both species were slowly acclimated to the low hardness and low pH conditions over the course of 5 days. Fathead minnows (< 24-hr old larval fish) were exposed in 7-day short-term chronic tests at three separate pHs (6, 6.5, and 7). Rainbow trout early-life stage (ELS) tests were initiated with eyed embryos and continued for 52-days at two separate pHs (6 and 7). Survival, growth, and hatching (trout tests) endpoints were assessed. In the fathead minnow tests, Cu toxicity decreased slightly as pH increased with 10% effect concentrations (EC_{10}s) of 10.2, 15.3, and 19.7 μg/L dissolved Cu at pH 6, 6.5, and 7, respectively. However, these differences were not statistically significant. In the rainbow trout tests, the most sensitive endpoint at pH 6 was wet biomass (EC_{10} of 28.2 μg/L dissolved Cu), while the most sensitive at pH 7 was mean day to hatch (EC_{10} of 38.8 μg/L dissolved Cu). Overall, this work indicates no or only a slight trend of increasing copper toxicity to fish with decreasing pH over the range of pHs tested.

MO078

Effect of salinity on silver absorption in hemolymph of the marine amphipod (Parhyale hawaiensis)

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The availability and toxicity of metals in the environment change at different salinities. Once some marine organisms, such as the amphipod *Parhyale hawaiensis*, are able to survive in a wide salinity ranges, is important to understand how the salinity can affect the absorption of metals, for example, silver. Silver’s release into the environment is becoming an environmental concern due to its large incorporation in nanomaterials. The aim of this study was to assess the silver absorption in hemolymph of *P. hawaiensis* at two different salinities. Also, we evaluated if copper could be used as an internal standard to normalize the silver measurements. Experiments were performed using adult organisms (8 months) individually exposed. The animals were acclimated to the test salinity for at least two weeks. The salinities tested were 30 and 10 and the Ag concentrations of exposure were 0; 5; 10; 25; 50 and 100 $\mu g\ L^{-1}$. After exposure, hemolymph was collected and weighted. Three pooled samples of 4 organisms were tested per salinity and per exposure concentration. The pooled hemolymph was diluted in 0.5 or 1 mL of HNO_3 0.05%. An AAnalyst 600 PerkinElmer Graphite Furnace Atomic Absorption Spectrometer was used for Ag and Cu determinations. For both salinities, silver concentrations in the hemolymph increased with the increase of Ag in water and it seems to be regulated, especially when the ion concentration is higher than 25 $\mu g\ L^{-1}$ in the water. Without using the copper as an internal standard, Ag concentrations for salinity 30 were higher than for salinity 10. Because copper concentrations in hemolymph of organisms at salinity 30 were significantly higher than those at salinity 10 (65±7 ng mg^{-1} and 50±5 ng mg^{-1}, respectively), when silver concentration data was normalized by copper, it was found no difference between silver absorption at salinity 30 and 10. The measurement of metals in hemolymph of such small organisms can be an interesting tool to evaluate internal dose. The use of copper as an internal standard seems to be an interesting way for normalizing the results.

MO079

Changes in trace metal concentrations and partitioning after the re-connection of a secondary channel to the main river channel during a floodplain restoration project

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In recent years, important efforts are being put into river restoration. Water quality is one of the factors determining restoration outcomes. The re-connection of disconnected secondary channels is likely to induce major changes in the physico-chemical conditions of these sites. Combined with the putative remobilization of legacy pollution in sediments, floodplain restoration is expected to affect trace metal fate and distribution. In this study, we tested the mid-term impact of the re-connection of a secondary channel to the main channel on trace metal concentrations, partitioning and accumulation in aquatic invertebrates. We measured Al, Ni, Cu, Zn, Cd and Pb in the water, sediment, suspended particulate (SPM) and deployed diffusive gradient in thin film devices (DGT) to measure labile fractions. We also collected aquatic invertebrates for metal analyis. Additionally, the invertebrate community composition before and after the restoration was compared. In the dissolved fraction, trace metal concentrations increased after restoration, trace metal concentrations in sediment and SPM decreased after restoration but sediment concentrations remained high compared to other sites of the same area. In three aquatic gastropod species, trace metal concentrations increased after the reconnection of the secondary channel. These results confirm the impact of restoration on trace metal concentrations and partitioning and suggest that this process should be considered in large scale restoration projects.

MO080
Fate, speciation, and bioavailability of vanadium in sediments
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Relatively little is known about the bioavailability and speciation of vanadium (V) in sediments despite its occurrence in aquatic environments and increasing V production and use. We studied reactivity, speciation, and bioavailability of V in sediments in laboratory mesocosms and field studies. Surficial sediments were collected from a river reservoir located in the drainage of a former mining area and contained elevated concentrations of vanadium (range: 240-1620 μg g-1) and other metals. Chemical analyses of sediments included V speciation (+4, +5), individual metals, total metal content, Fe/Mn-oxyhydroxide V binding, organic carbon, acid volatile sulfide, pH, and Eh. Chronic (28-day) sediment toxicity tests were conducted on Hyalella azteca using field-collected sediments and sediments spiked with V. Additional toxicity testing included a water level fluctuation experiment with non-spiked sediments to investigate V redox and porewater/sediment interactions. For field validation, sediment trays were reciprocally transplanted between reference and contaminated sites and analyzed for macroinvertebrate colonization. Relative growth rates of H. azteca did not significantly correlate to whole sediment or porewater V concentrations or bioaccumulation of any metal. A simulation of reservoir drawdown showed relatively low V concentrations in porewater which did not affect H. azteca. While V body burden on H. azteca was higher for contaminated than reference sediments, there were no associated acute or chronic effects. Increased Zn in porewater was found to exceed the USEPA threshold for chronic and acute toxicity to freshwater organisms. H. azteca body burden and growth rates were negatively correlated for Zn ($p < 0.001$), but not for V. Despite sediment chemistry differences between sites, metrics for benthic communities on transplanted sediments were similar. Transplanted sediments were colonized with pollution-sensitive benthic organisms, suggesting benthic community structure was not impacted by site sediments containing elevated levels of V, Zn and other metals. Our studies show V bioavailability and environmental risk is low despite relatively high sediment concentrations, and V is less susceptible to redox changes associated with water level fluctuations than other more labile metals. Although Cr, Ni, and Zn all exceeded sediment PECs, only Zn appeared to have an effect on measured endpoints (reduced amphipod growth).

MO081
Bioaccumulation of mercury in river sediment and freshwater tilapia Oreochromis mossambicus
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One of the greatest threats to freshwater fish and a major factor demanding an intensive ongoing management is mercury (Hg) contamination. The purpose of this paper is to investigate Hg concentrations in freshwater tilapia *Oreochromis mossambicus* and river sediment from different regions of Taiwan. Here we showed that large amount of Hg accumulated in sediments ranging from 0.16 - 43.56 and 0.006 - 1.70 mg kg^{-1} ww for Hg(II) and methylmercury (MeHg), respectively, compared with those in water with the range of 2.60×10^{-6} - 7.32×10^{-4} and 8.57×10^{-8} - 2.41×10^{-5} mg L^{-1} for Hg(II) and MeHg, respectively. To obtain the time-course concentrations of different Hg species in water and sediment, a water-sediment dynamic model was developed. Our results showed that Hg(II) was the predominant species in both water and sediment, accounting for nearly 96% of total Hg (THg). These results support that sediment is the major reservoir for Hg in aquatic environments. We further investigated the internal exposure in different tilapia tissues/organs from intake of river water and sediment. We found that tissue/irgan burdens would reach equilibrium before 180 days of exposure in all rivers. Among all exposed doses tissues/organs, kindey has the highest internal exposure doses of Hg(II) ranging from 0.021 - 0.135 ug g^{-1} ww. In constrast, muscle had the lowest internal exposure doses of 0.0001 - 0.0003 ug g^{-1} ww Hg(II), indicating that Hg levels in muscle might be well below levels considered at risks for human consumption based on regulation from Taiwan FDA. We also incorporated the sediment concentration data of THg and the sediments of transport parameters with initial conditions into the water-sediment model to estimate the steady-state concentrations of Hg(II) and MeHg in study rivers. We showed that our water-sediment model is simple yet robust enough to be used to address the complex transformation and distribution of different Hg species in water and sediment.

MO082
Influence of trace metal pollution on benthic macro-invertebrates in the Berg River Catchment, South Africa
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Benthic macroinvertebrates are known to be influenced by several physico-chemical and biological factors. Sediment Parameters such as sediment particle size, organic matter content, pH, nutrients, anthropogenic pollution, are some of the factors influencing benthic macroinvertebrate communities. Sediments are known to form a vital and integral part of the aquatic environment. With the close association with the overlying water in the aquatic ecosystem, research has indicated that sediments act as a potential sink for various pollutants. Pollutants settle with particulate matter from the water column onto sediment, thereby allowing hazardous and toxic metals to accumulate in sediments that can be extremely harmful for the aquatic environment. The emission of heavy metals from wastewater effluent discharges and other industrial activities in the South African environment has been receiving considerable attention, due to the potential threat to human health and the environment. These metals have the potential to accumulate in sediment and biota, posing a serious threat to aquatic ecosystem health. It is further known that some of these elements are toxic to living organisms even at quite low concentrations. Research has further shown that aquatic macro-invertebrates form an integral part of the diet of freshwater fish and can be considered an important step in the aquatic food chain, playing an important role in the trophic transfer of pollutants, e.g. trace metals. The present study considered the bioaccumulation of trace metals such as cadmium (Cd), lead (Pb), copper (Cu) and chromium (Cr) in freshwater invertebrates. The results obtained have shown that the concentrations for Cu (24.65 mg/kg) and Pb (30.13 mg/kg) were highest in the sediment, especially in the wet season. Macroinvertebrate (*Libellulidae spp.*) results showed highest concentrations of Cu (52135 μg/kg) and Pb (9835 μg/kg) in the dry season. The results further indicated that the metal concentrations are influenced by anthropogenic activities at the sampling sites, with the benthic invertebrates showing differences in the heavy metal concentrations accumulated, taxon richness, number density and biodiversity for the different sampling sites evaluated in the Berg River, Western Cape Province, South Africa.

MO083
Biogeochemical distribution of Cr, Cu and Zn in a riverbed contaminated by tannery effluents and pig farms
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Guadalentín River (SE Spain) has been affected by tannery industries and pig farms, where their effluents containing Cr, Cu and Zn were spilled until 2003. The main objectives of this research were to evaluate the contamination and the spatial distribution of Cr, Cu and Zn in the sediments of the riverbed area and the influence of sediments properties in the behavior of metals. A sediment sampling was carried out in a stretch of 1500 m of the dry riverbed, from 0 to 100 cm deep, in order to determine the degree of Cr, Cu and Zn pollution and the influence of sediment properties. Total, soluble, and exchangeable Cr, Cu and Zn were analysed in sediments using graphite furnace atomic absorption spectrometry. A biogeochemical characterization of the riverbed sediments was performed with the aim of evaluating the influence of sediment properties to the contents of total metals. Total Cr concentration was the highest in the riverbed (11–11099 mg kg^{-1}) up to 100 cm deep, exceeding in almost all the study stretch the background and the generic reference values (> 66 mg Cr kg^{-1}). The largest degrees of sediment pollution (over 10.000 mg Cr kg^{-1}) was found at 20–50 cm deep, at the first 600 m east of the study area, and in the last 300 m of the studied area. This reveals that the Cr concentration in sediments is relatively higher near the discharge point of the tannery facilities. Total Cu concentration exceeded the generic reference levels (> 32 mg Cu kg^{-1}) up to 100 cm deep in the first 500 meters of the study area. With respect to total Zn concentration, there is contamination (> 105 mg Zn kg^{-1}) in the first 500 meters of the study area between 0-20 cm depth. The main conclusion was that the riverbed sediment pollution by Cr, Cu and Zn was caused by an anthropogenic activity (tannery industry).

MO084
Metal mobilization regulates pollution levels in anthropogenic impacted estuarine sediments
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Metals are problematic pollutants in estuaries where contamination from multiple sources can undergo remobilization driven by physical and chemical processes. Such mobilization changes sediment contaminant signatures, which hampers the identification of metal sources and misleads interpretation of potential environmental impacts. Thus, understanding the metal mobility in estuarine sediments is essential to improve identification of pollution sources and their accountability for effects. This study aimed to evaluate metal mobilization under controlled conditions and to investigate the impact of metal mobilization on pollution levels in sediments. Laboratory experiments on metal mobilization were combined with field assessment of metal pollution to understand metal behavior in an intertidal flat with multiple contaminant sources. Sediments from a saltmarsh adjacent to a coastal landfill, Yacht club and docking area (Canvey Heights Park, Thames Estuary, UK) were exposed (24 h, N = 96, 20 °C) to water at various salinity, pH, and redox in the laboratory. The resultant slurry was analysed for pH, redox potential, and salinity. Major cations, Fe^{2+}, and filtered metal concentrations were analysed in the leachate and sediment. An empirical regression model was developed from the correlation between sediment physico-chemistry and metal

mobilization to water phase. Salinity, pH and redox had a significant effect on metal mobilization ($p < 0.001$), e.g. depending on the combination of these parameters Fe leaching could be increased up to 1000-fold. Landfill proximity poorly explained surface (5 cm) and subsurface (up to 4 m) metal spatial distribution. However, physicochemical parameters explain up to 97% of geochemically normalized sediment metal concentrations. In the field, organic matter and pH were the parameters correlated with most metals at the sediment surface. At subsurface, major cations (i.e. Ca, Na, Mg and K) were more important. Applying the empirical model obtained in the laboratory to geochemical conditions of the saltmarsh, it was possible to demonstrate that Fe mobilization has strong influence on the fate of this (and potentially other) metal in that area ($r^2 = 0.42$, $p < 0.001$). Thus, present results highlight the importance of metal mobility to control sediment pollution and estuarine fate of metals. Further studies are required to investigate whether other estuarine systems present similar high metal mobilisation as presented here.

MO085
Aquatic bioaccumulation of lanthanides and yttrium: a bibliographical review in a regulatory context
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Under several international regulations, including REACH Regulation (EC) No 1907/2006, substances should be assessed for environmental hazard classification. Data on bioaccumulation are of key importance in this process because they are considered, together with degradation and ecotoxicity information, to assess the chronic hazard to the aquatic environment. It has been often highlighted that bioaccumulation of inorganics (including metals) follows a different paradigm relative to organics; and this, for several reasons: •Non applicability of Kow concept, •Influence of environmental conditions, •Inverse relationship between bioaccumulation values and concentrations into water, •Existence of internal regulatory mechanisms. Nevertheless, the concept that a substance may bioaccumulate is important for these substances too and need to be assessed even if the thresholds and underlying mechanisms cannot be considered in the same way than for 'classical' organics. Evaluation should be thus performed on a case-by-case basis and expert judgment can be used to conclude that a substance is unlikely to pose a risk to predatory organisms or humans exposed via the environment either: (i) based on the absence of food web biomagnification and information showing that organisms in higher trophic levels are not more sensitive than those in lower trophic levels after long-term exposure, or (ii) because it is an essential element and internal concentrations will be well-regulated at the exposure concentrations anticipated (ECHA Guidance R7c, 2014). Such an expert judgment was developed for lanthanides and yttrium (known together as rare earths, RE). A bibliographical review was conducted by considering all studies on bioaccumulation in aquatic organisms; resulting in a database composed of *ca.* 60 publications (1964-2016) reporting laboratory and field data on all elements and several trophic levels. The main conclusions of this review are the followings: •Different trophic levels present internal regulatory mechanisms. •A considerable decrease of bioaccumulation was observed when ascending the trophic levels; this being obvious when comparing data in fish to those in lower trophic levels. •RE do not biomagnify through the aquatic food web. Based on this pool of evidences, it was concluded that RE are unlikely to biomagnify in predatory organisms or humans exposed via the environment.

MO086
Bioavailability of metals: environmental compliance check via PNEC-pro V6
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Environmental quality standards for heavy metals in sediments and surface waters were developed to protect the ecosystem from adverse effects. These quality standards are generic and therefore apply to all surface waters. The importance of explicitly considering bioavailability - dictated by the chemistry of the surface water - has been recognized for some time. Biotic Ligand Models (BLMs) are the practical modelling implementation to achieve bioavailability-based quality standards. However, the major barrier to a widespread, practical use and implementation of BLMs is the conceptual complexity of the approach, requiring advanced chemical speciation calculations and normalization procedures with toxicity data. Moreover, BLMs may require a large number of (measured) input parameters, some of which are not readily available in standard monitoring programmes. To overcome these difficulties, the software *PNEC-pro* was developed. *PNEC-pro*V6 is a user-friendly, state-of-the-art tool for professionals dealing with the assessment of surface water quality. It calculates local, watertype-specific no-effect concentrations (PNEC) of copper, nickel, zinc, and lead based on Biotic Ligand Models (BLMs). Local PNECs are used for compliance checks in higher-tier risk assessments. *PNEC-pro* uses validated, multi-parameter transfer functions that are based on large up-to-date toxicity datasets and a broad range of water chemistry data covering almost all water types from the Water Framework Directive. The software uses routines that select functions with the highest reliability, based on the input parameters that are entered by the user. They replace complicated full-BLM procedures and increase the applicability for "routine" water type-specific risk assessments. *PNEC-pro* generates local PNECs, Risk Characterization Ratios (RCR), and the probability that local concentrations exceed the local PNECs. Frequency distributions of monitoring parameters and PNECs are generated and visualized as graphs, and a statistical summary of the dataset is produced. *PNEC-pro* is endorsed by the Dutch Ministry of Infrastructure and the Environment, and has been implemented in legal frameworks.

MO087
Bioavailable EQS for Lead for European Surface Waters: An Update on DOC- and BLM-based Approaches
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The current Environmental Quality Standard (EQS) for lead (Pb) under the Water Framework Directive (WFD) in the EU uses a bioavailability approach that only takes account of dissolved organic carbon (DOC) to derive site-specific EQS for a freshwater ecosystem. While DOC is a major water chemistry factor to mitigate Pb toxicity in freshwater, other parameters, specially water pH and calcium also have influence on Pb bioavailability. A chronic biotic ligand model (BLM) that accounts for all of these factors were developed for Pb from a large array of ecotoxicological data and applied to derive EQS for selected European freshwater scenarios: Ditches (NL), River Otter (UK), River Teme (UK), River Rhine (NL), River Ebro (ES), Lake Monate (IT), and neutral acidic lake (SE). The ranges of pH (6.7–8.2), DOC (2.5– 12.0 mg/L), and Ca (8.7–60.1 mg/L) in these scenarios cover a wide range of water chemistry conditions to represent typical cases of bioavailability for European surface waters. Based on the parametric best-fitting SSDs (species sensitivity distributions) that were normalized with the chronic BLM, the calculated 5th percentile hazardous concentrations of Pb (i.e., ecological threshold concentrations or EQS protecting 95% of freshwater species) for the seven selected European freshwater scenarios were between 6.3 μg Pb/L and 31.1 μg Pb/L. On the other hand, the EQSs calculated with the DOC equation used under the WFD vary between 3.0 μg Pb/L and 14.4 μg Pb/L for the same scenarios. The reference EQS (1.2 μg Pb/L) that represents a situation of high bioavailability (DOC = 1 mg/L) and is currently used in the EU is significantly lower than those for the above scenarios, highlighting the protectiveness of the current bioavailable EQS for Pb. A comparative analysis of DOC- and BLM-based approaches for a wider range of European surface waters will be provided.

MO088
A new field method for assessing chromium and nickel pollution immobization in soils of tropical urban low lands
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The occurrence of heavy metal pollution in the environment and its management is a major challenge in many countries worldwide. This study investigated the retention ratios (RR) of Cr and Ni in soils in Yaoundé, Cameroon to propose simple solution for pollution mitigation. Soils investigated were sampled in five (05) representative profiles. A simple indigenous water conservation system was temporary prepared around each sampling site (a simple contour slope wall constructed with stones across the slopes thereby intercepting the surface runoff and maximizing the accumulation of pollutants transported by the runoff at that point) during rainy season. Thereafter, soil profiles were dug (up to the water table) after rainy events and total water infiltration. Soil samples were collected in the surface horizons (0-20 cm) and at the bottom of the unsaturated zone of the soil profiles (with variable depth). In each profiles, 5 samples were collected at surface horizon and 5 samples at the bottom of the unsaturated zone. samples were air-dried and ground to pass through a 2 mm sieve and different soil- water extract was prepared for metal analysis. By soil digestion method with diacid, total Cr and Ni were extracted and determined by atomic absorption spectrophotometry (AAS). Results showed decreasing trend (from the soil surface to the bottom of verdoze zone) of heavy metal concentrations, regardless the sampling station. The retention ratios (RR) of the Rhodic ferralsol, Xanthic ferralsol and Mollic gleysol (2) were very high regardless of the heavy metal examined (*RR* > 80%), except for Ni where it was found equal to 75% in Rhodic Ferralsol. In contrast the retention ratio values of the Plenthic gleysol and the Mollic gleysol (1) were relatively low, regardless of the heavy metal concerned. Therefore, *RR* was generally < 30%: about 10% for Plenthic gleysol (in case of Cr); 20% for Mollic gleysols (1) (for Cr and Ni). High retention ratio soils could be used at low cost in containment technologies in the construction of engineered solid waste dumpsites to restrict the movement of heavy metals in the environment.

MO089
Principal component analysis for choosing of barrier materials for groundwater treatment
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Industrial discharge of organic and inorganic pollutants has resulted in contaminated groundwater worldwide. As groundwater contaminants may have serious health consequences, groundwater treatment techniques that can handle multiple organic and inorganic contaminants are needed now and for the

foreseeable future. Six materials were selected for this study: activated carbon; fly ash; lignite; torrefied material (pyrolysed pine stemwood; peat; cast iron powder.Equilibrium adsorption experiments were performed with two types of solution: 1) groundwater taken from an industrial area in northern Sweden with a DOC concentration of 770 mg L-1, and 2) a reference Nordic fulvic acid (FA) solution (International Humic Substance Society, Norway) with a DOC concentration of 300 mg L-1. Both solutions were spiked with a stock solution of organic pollutants. The FA solution was additionally spiked with a heavy metal stock solution. Assessment of the adsorption characteristics of sorbents was then carried out using the Freundlich model. Principal components analysis (PCA) was used to analyze the constants of the Freundlich equation to reveal similarities and differences between individual samples, and to find relationships among the measured parameters. Five principal components (PCs) were calculated that condense 82% of the variation for n coefficient in the descriptor set. From the analysis of the projection of elements on the plane spanned by PC 1 and PC 2, it can be concluded that the variables that make the greatest contribution to the creation of the first principal component (PC 1) are the sorbents that remove organic pollutants. The greatest contribution to the second principal component (PC 2) are the sorbents that remove heavy metals and DOC. We also can derive separate group included Ba and some organic pollutants (dichlorobenzenes, methylated polyaromatic hydrocarbons, aliphatics C22-C28). We can conclude that fly ash, iron (aerobic conditions) and activated carbon are promising materials for DOM removing. For metal removing the most effective were fly ash, lignite and iron (aerobic conditions). Carbon based material (activated carbon, lignite and peat) and fly ash were the most effective in organic contaminants removal. Aeration seems to increase the removal efficiency of iron materials, because of formation of a porous rust layer.

MO090

Application of Alginate-Clinoptilolite Beads for the Removal of Heavy Metals

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Heavy metals are widely used in different industries during manufacturing processes. These metals are known as toxic materials to human body and other livings at considerably low concentrations. For this reason, heavy metals are subjected to treatment by various processes before discharge into water bodies. Adsorption is one of them which is easy to apply, cheaper depending on the adsorbent used and supplies selective removal of different metals. In recent years, natural adsorbents are getting attention and alginate might be an example. Alginate, a biopolymer composed of mannuronic and guluronic acid, was found to be effective for heavy metal adsorption particularly due to carboxylic acid groups on the polymer backbone. Further studies continue to improve heavy metal uptake capacity of alginates by combining with other materials having ability to capture metals. In this study, clinoptilolite is selected and used to form alginate-clinoptilolite beads. Clinoptilolite is a natural zeolite which is effective in heavy metal uptake by ion exchange. Preliminary experiments showed that alginate-clinoptilolite beads were successful for the removal of copper ion. The maximum adsorption capacity was calculated as 131.6 mg Cu^{2+}/ g alginate-clinoptilolite beads according to the Langmuir isotherm model. The aim of the current work is to investigate the removal of heavy metals from a synthetic solution containing mixture of metals, Cd^{2+}, Cu^{2+} and Pb^{2+}, by batch adsorption experiments using alginate-clinoptilolite beads. Adsorption kinetics will be examined and the results will be evaluated for optimum conditions to achieve the maximum heavy metal uptake by giving special attention to the ratio of alginate to clinoptilolite in composite beads and the size of clinoptilolite.

MO091

Effect of mineral supplements on lead bioaccessibility in livestock: An integrated laboratory and field-based approach

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Lead (Pb) mining has contributed to the extensive release of Pb in nature for centuries. In old mining districts, nowadays largely occupied by livestock and hunting estates, the concentrations still present in the affected areas may constitute an environmental and health risk. Because Pb is considered one of the most toxic metals, it is necessary to explore how to prevent or reduce exposure. Many essential elements, such as calcium (Ca) and phosphorus (P), alter Pb solubility and absorption in the digestive tract. Here, we studied the effect of several commercial mineral supplements on Pb bioaccessibility in order to prevent/reduce absorption in ungulates inhabiting mining areas. In a first step, we compared the effect of 12 different types of commercial mineral supplements on Pb bioaccessibility from contaminated soils in an in vitro ruminal stomach-intestine system. Results showed a significantly lower level of bioaccessible Pb in a mineral supplement with a high content of Ca and P than in the rest of salts, both in stomach an intestine phase. Moreover, a significant reduction in the level of Pb bioaccessible was detected for mineral supplements containing molasses in comparison to those without molasses. In the stomach phase, significant negative correlations were observed between Pb and the levels of Ca, P, magnesium (Mg), and manganese (Mn) present in the mineral supplements. In the intestine phase, negative correlations were found between Pb and the levels of P, iodine (I), and copper Cu. With these results, a second step was performed on a sheep farm located in an old mining area where animals graze year round on pasture surrounding abandoned Pb mines. Fifty female sheep were divided in two similar groups, and located in two different plots with similar Pb concentrations in soils. Only one of the plots was supplemented with a commercial mineral supplement: the selected as the best one after the in vitro simulation. The rest of the conditions were the same for both groups. Blood samples were collected three time during the field experiment, every 20 days. Results showed that blood Pb levels in sheep exposed to mineral supplementation decreased throughout the experiment, while Pb levels increased in those not supplemented. Mineral supplements enriched with Ca, P, Mn, Mg, I and Cu would potentially decrease Pb bioavalability and be used as a palitaive cost-effective measure to reduce Pb exposure of livestock and wildlife in Pb contaminated areas.

MO092

Bioaccessiblity of selected trace elements in indoor dust from Istanbul-Turkey

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Indoor dust might play an important role on exposure of human to inorganic and organic pollutants via inhalation, dermal contact and ingestion of dust. Heavy metals such as lead (Pb), chromium (Cr), cadmium (Cd) are abundant in indoor dust and are well in excess of crustal and soil levels. Infiltration of outdoor air containing suspended particulate matter (PM) and track-in of soil adhering to footwear are the main migration pathways of inorganic contaminants to indoor environments. Over the past years, there has been an increased concern on the exposure of people, especially of vulnerable groups such as children, to indoor contaminants in order to assess the health effects. Although there exist several studies on the detection of levels of trace elements levels in indoor dust, studies on the bioaccessibility (or solubility) in different stages of gastrointestinal environment is limited. Early studies on the assessment of bioaccessibility in dust have been based primarily on dilute HCl extractability with aid of pepsin. However, such an approach or assumption of 100% bioavailability of trace metals in indoor dust appear to overestimate human exposure to these contaminants via indoor dust. To the best of knowledge, biaoccessibility tests have been to very limited number of indoor dust samples with a focus on one or two trace elements. Therefore, in this study, we aim to investigate biaccebility variations of heavy metals in indoor dust using solutions that simulates human gastointestinal system. The Dutch National Institute for Public Health and the Environment (RIVM) in vitro bioaccesibility method is applied on 50 indoor dust samples and bioavailable fractions of cadmium (Cd), mercury (Hg), Pb, vanadium (V), Cr, Cd, manganese (Mn), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn) and Arsenic (As) is determined. Average concentrations ranged between 0.450 to 620 μg/g depending on the trace element. The largest BA process occurs in gastric juice floowed bu duodenal part and mouth once heavy metal adsorbed dust is ingested by human. Total BA value ranged between 45% and 65% depending on trace element and based on these results, an assumption of 100% bioaccessibility of heavy metals in indoor dust might lead overestimation of exposed concentration. Rasults of the study provides a useful refining of estimayes of bioaccessibility of a wide range of metals detected in indoor dust and can be used to develop assimilation models.

MO093

Levels of Selected Heavy Metals in Butter Samples Collected from Different Provinces in Turkey: An assessment of Human Exposure via diet

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Trace elements are non-biodegradable and non-terhodegradable and considered as serious inorganic pollutants because of their toxicity on living organisms. The heavy metals enter the human body through inhalation and ingestion whereas intake via diet depends upon food habits. The quality of the dairy products is directly related to the concentration of trace elements. Since diet is the most significant human exposure pathway to Cd (II), Pb(II) and Cu(II), the tolerable lead consumption per week was reduced from 0.05 mg kg^{-1} body weight to 0.025 mg kg^{-1} by the Joint FAO/WHO Expert Committee for Additives and Contaminants (JECFA) in 1993, whereas recommended daily intake of cadmium and copper is 7 mg kg^{-1} and 0.5 mg kg^{-1} body mass, respectively. Abou-Arab (1991) reported that butter is more susceptible to trace metal accumulation than milk and cream. In Turkey, although there are studies on the trace elements compositions of edible oils, raw milk, ayran (yoghurt drink), white cheese, kashar cheese samples, to the best knowledge of the authors, there is only one study on butter (Dervisoglu et al., 2014) and yet these studies focuses only a given region of the country. Therefore, to the best knowledge of the authors, there are no studies investigating heavy metals content of butter samples on a countrywide scale. The main aim of this study was to assess the heavy metals pollution in different regions of Turkey by using local butter samples which were collected in the same time span. Among the average concentrations of target trace elements, Zn showed the highest concentration in samples followed by Cr, Pb, Cu, Hg, Cd and As. Provisional maximum tolerable daily intake (PMTDI) and the dietary reference intake (DRI) levels for Cu are 50–500 $\mu g\ kg^{-1}$ BW and 700–900 $\mu g\ kg^{-1}$ BW, respectively and it seems that the estimated daily intake (EDI) of Cu (0.007 $\mu g\ kg^{-1}$ bw) is lower than this established values. Turkish Food Codex sets a limit value of 0.02 mg/kg Pb for milk and dairy products and average value of Pb was higher than limit value.

MO094

Assessment of trace element contents in water, sediment and food web components along the ultramafic shoreline of Lake Ohrid (Albania)

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Lakes located in mining areas can be heavily contaminated by metals, which afterwards may be accumulated in tissues of aquatic organisms and transferred up food webs. The transboundary lake Ohrid, shared between Albania and Macedonia, is one of these lakes. Its Albanian side is naturally rich in minerals of ferrochromium, ferronickel, chromites which were historically extracted and processed until the early nineties. However, several ore dump sites remain near the shore of the lake, thus representing one potential input source of metal elements. Several creeks flowing accross ultramafic areas, also contribute to metal inputs into the lake. As aquatic organisms may suffer from this contamination, there is a need to monitor trace metal levels in Lake Ohrid and their distribution, as well as their accumulation and potential transfer in benthic-pelagic food webs in order to highlight a potential ecotoxicological risk for predators or humans. Samples of surface water, sediment and biota from pelagic and benthic zones (i.e. phytoplankton, zooplankton, periphyton, macrophytes, gastropods, bivalves, gammarids and fishes) were collected at four sites and analyzed for eight elements (Cd, Co, Cr, Cu, Fe, Mn, Ni and Zn) by atomic absorption spectroscopy. Low levels of trace elements were quantified in surface waters, but sediments contained high levels with concentrations reaching 93.8 mg/kg for Co, 345.1 mg/kg for Cr, 553.8 mg/kg for Ni, 49.9 g/kg for Fe and 872.9 mg/kg for Mn, with the highest values found near creek inflows or mineral dump sites. Among all sampled food web components, phytoplankton and periphyton at the basis of food chains, accumulated high metal concentrations, but no apparent trophic transfer to invertebrate consumers was highlighted. At the top of the lake food web, the endemic Ohrid trout *Salmo letnica* accumulated low levels of metals in its muscles. The present results suggest that biomagnification of the selected trace elements is unlikely in Lake Ohrid.

MO095

Analysis and risk characterization of arsenic species in food supplements based on clay.

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15 clay containing food supplements (FS), in which often also natural ingredients such as plants were present, were collected on the Belgian market and analyzed for total arsenic (As_{tot}), inorganic arsenic (As_i), AB (arsenobetaine), DMA (dimethyl arsenate) and MA (monomethyl arsenate). As speciation analysis was conducted by HPLC-ICP-MS, by means of a method which is commonly used for As speciation analysis in food (cfr method CEN16802; further referred to as 'food-method'). In addition the bioaccessible As fraction in these samples was determined by use of the UBM protocol, an in vitro simulation of the gastro-intestinal tract (Denys et al., 2012). Total As in the samples was analyzed by ICP-MS. Clay containing FS form a particular group of food supplements in relation to potential As toxicity, because a large fraction of arsenic in these samples is expected to be present in the most toxic inorganic form (Asi).. In terms of risk, the most important question likely is not which species are present, but rather what the bioaccessibility is of Asi. The results revealed that bioaccessibility of As(i) varied between 7.7% and 50.9 % among all clay based samples, and that -overall- the food method was only a poor predictor of the bioaccessible Asi fraction despite the significant relationship. In a second step an exposure and risk assessment of As for FS consumers was performed. Exposure was calculated for each FS by multiplying the concentration of these compounds with the maximal recommended dose. Risksrelated to the intake of As species were evaluated by comparing the calculated exposure to the reference values suggested by ATSDR(2007), JECFA(2011) and EFSA(2009). If the reference value was a MRL-value, the conclusion 'concern' was given if the calculated exposure was higher than the MRL value. If the reference value was a BMDL-value, then a 'Margin-of-exposure' (MOE=BDML/exposure) was calculated. The conclusion 'concern' was given when the MOE< 100. In relation to Asi exposure we worked with the assumption 'AsTot = total inorganic As'; and with the bioaccessible As. Regarding MA and DMA no (sub)chronic risk was present, and no risk for acute toxicity of Asi was detected. Depending on the reference value used, the conclusion 'concern' for chronic toxicity, was given in 14/15 when using the total (inorganic) As concentration and in 11/15 samples when using the bioaccessible As concentration.

MO096

Modelling networks of causal relationships in stress ecology using Structural Equation Model: an example linking fate and impact of metals in contaminated soils

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Testing the complex hypotheses reflecting the effect of stressors in natural conditions needs advanced multivariate analyses. This study presents a promising multivariate analysis (structural equation model: SEM) able to model networks of causal relationships and conceptual (latent) variables reflected by several observed variables. The aim is to show the interest of SEM for analysing monitoring data based on the example of the bioavailability of metals to earthworm. The concept of bioavailability perfectly illustrates the key features of SEM relevant for field data analysis (e.g. causal relationships and numerous variables supposedly reflecting bioavailability). A SEM reflecting the causal assumptions: the more metals are available in the soil the more they enter the organism, and an effect can only appear if metals have entered the organism, was tested. This definition involves 3 sub-concepts: 1) *environmental availability*: available metals in soil, measured by e.g. chemical metal extractions, 2) *environmental bioavailability*: metal absorbtion by the organism, measured by internal metal concents, and 3) *toxicological bioavailability*: internal metals leading to effects, measured by biomarkers. In the SEM each sub-concept was a latent variable reflected by several observed variables. The SEM was tested based on a large dataset for a relevant earthworm species: *Aporrectodea caliginosa* exposed to 31 field soils. The first interest of SEM was the possibility to test for causal relationships. For Cd, the SEM was supported by the data and suggested that earthworms may not be exposed only to readily available metals (e.g. dissolved Cd content in soil), but possibly also to metals bound to soil particles ingested by earthworms. Another key feature of SEM was to model unmeasured concepts reflected by several indicators. The observed variables of a latent are correlated in SEM. This is an important advantage of SEM, valuable when analysing monitoring data and dealing with the issue of inter-correlations between e.g. physico-chemical variables. This study shows that SEM can elucidate the causal links involved in the exposure-effects of chemical stressors. The potentialities of this modelling framework applied on large datatsets such as monitoring data are tremendous. The ability to model networks of interacting components will help refining our causal understanding of the effects of stressors on biodiversity and ecosystem functioning in natural environments.

MO097

Synthesis, characterisation and application of chitosan beads-supported Fe-Ag bimetallic nanoparticles for the removal of cadmium from wastewaters

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Nowadays, water is the most important concern of this century, it is our common future and is important to preserve and guarantee this natural precious capital. However, the supply of good quality water becoming increasingly difficult in view of large scale pollution caused by agricultural, domestic and industrial activities. Many technologies including coagulation, membrane process, dialysis, foam flotation, osmosis, photocatalytic degradation and biological methods have been employed for the removal of toxic pollutants from water and wastewater. These technologies are effective but have some disadvantages such as expensive equipment, high operational and maintenance, high energy requirements, generation of toxic residual metal sludge and incomplete metal removal. On the other hand adsorption offers high efficiency, cost-effectiveness, easy handling and recovery of metals and other adsorbed species. Heavy metals are often found in wastewaters and the removal of these inorganic pollutants using bimetallic iron-based nanoparticles is still unclear. In this study, bimetallic iron-silver nanoparticles was chemically synthesized and impregnated into chitosan to form chitosan bimetallic iron-silver nanoparticles (CS/Fe-AgNPs) to remove heavy metals from wastewaters. The above CS/Fe-AgNPs beads were characterized using XRD, SEM, TEM, UV-VIS and FT-IR techniques. In this study, chitosan iron-silver nanoparticles beads have been successfully prepared and it's efficiency in the removal of Cd(II) under ambient temperatures has been evaluated. The removal rate of total Cd(II) from actual wastewater was 91.4%. Furthermore, the

monolayer adsorption capacity of Cd(II) based on the Langmuir model was measured to be 90 mg/g. Results were satisfactory when employing the adsorbent for removal of Cd(II) from wastewater samples.

Modelling and monitoring of pesticides fate and exposure in a regulatory context (P)

MO098

Sorption-desorption of indaziflam in agricultural soils amended with different biochars

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The many advantageous properties of biochar have led to the recent interest in the use of this carbonaceous material as a soil amendment. However, there are limited studies dealing with the effect of biochar on the indaziflam (*N*-[(1*R*,2*S*)-2,3-dihydro-2,6-dimethyl-1*H*-inden-1-yl]-6-[(1*R*)-1-fluoroethyl]-1,3,5-triazine-2,4- diamine) behavior applied to crops. This study aimed to characterize the effect of amending soils with biochars derived from soybean stover, sugarcane bagasse, and wood chips derived biochars, or same raw feedstocks, on the sorption-desorption of indaziflam applied to soils from three Midwestern U.S. states, a silt loam (Minnesota - MN and Pennsylvania - PA) and a silty clay loam (Illinois - IL). We used the batch-equilibrium methodto determine the concentration of ^{14}C-indaziflam (radiochemical purity = 97.9%, specific activity = 3.96 MBq mg^{-1}) in a pseudo-steady state with soil and biochar-soil systems at 10% (w/w). Based on the partition coefficient $K_{d\ (sorption)}$ values, indaziflam was considered relatively low sorption herbicide to the unamended three soils, with 1.46, 1.54, and 2.48 L kg^{-1} to MN, IL, and PA, respectively; and $K_{d\ (desorption)}$ = 5.17, 5.61, and 4.65 L kg^{-1}, respectively. Indaziflam sorption increased up 18 times in all amended soils with biochar as compared to the unamended soil and desorption increased up 6 times. Biochar produced from wood chips feedstock was the most effective biochar that we assessed for reducing (89-93%) and desorbing only 2-5% of the aqueous herbicide concentration. Therefore, the composition of the biochar in the soils amended can play an important role in the practical remediation by sorption-desorption of indaziflam, because these biochars reduced the herbicide concentration. Acknowledgement: São Paulo Research Foundation (FAPESP).

MO099

Glucose mineralization in soils of contrasting textures under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture

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Microbial adaptation in soil may occur in surface soil under agricultural uses exposed to herbicides. However, little is known about herbicide mixture effects in the soil, especially in tropical regions like Brazil. The aim of this study was to evaluate glucose mineralization in soils of contrasting textures (sandy clay and sandy loam) from areas cultivated with maize under application of S-metolachlor, terbuthylazine, and mesotrione alone and in a mixture. The methodology was established according to the soil microorganisms: carbon transformation test with ^{14}C-glucose solution (D-[U-^{14}C] glucose) in biometric flasks. After the addition of ^{14}C-glucose, the amount of ^{14}C in cumulative CO_2 of microbial respiration was measured several times during the 28-day incubation. A kinetic assessment of mineralization rates was done to compare different treatments. For unamended soil – control (without herbicide), microbial activity followed similar behavior to amended soil with herbicides in total $^{14}CO_2$ released and accumulated, ranging from 23 to 27%. Overall, mineralization constant rate (k) values for all treatments were also similar, with an average value of 0.0038% CO_2 d^{-1}, consequently mineralization half-life times (MT50) were from 173 to 198 d. Microbial respiration for all treatments was slightly higher in the sandy clay compared with sandy loam soil; although soil samples with application of herbicides (alones and in a mixture) did not have decreased basal microbial respiration or mineralization rate of glucose. Acknowledgement: São Paulo Research Foundation (FAPESP).

MO100

Estimation of DegT50matrix values from field dissipation studies: measured versus simulated soil moisture and soil temperature parameters

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To generate normalised DegT50matrix values based on field soil dissipation studies, the soil moisture and soil temperature are recorded on daily basis during the study. The climate values are subsequently used to "normalise" the observed decline in bulk soil to the standard temperature and moisture conditions of 20°C and pF2. Due to technical problems or other issues the required information on climate data might be missing. As described in FOCUS kinetics (2006), pesticide leaching models which simulate the concentration of pesticides in soil at different depths (e.g. PEARL) can be used to simulate the missing values based on other available climate parameters, like daily maximum air temperature, daily minimum air temperature, sum of daily precipitation and sum of daily global radiation. Two possible data sources for climate data with daily resolution could be used: 1) NASA provides a global coverage of the required parameters on a 1° latitude by 1° longitude grid and 2) the MARS (Monitoring Agricultural ResourceS) database from JRC provides data (for Europe) based on a 25 x 25 km grid. In the current investigation of soil dissipation for six trial sites across Europe the soil moisture and temperature will be estimated with FOCUS-PEARL 4.4.4 based on climate data provided by NASA and JRC. For each trial site a specific PEARL scenario will be created based on the available soil properties. The hydraulic pedotransfer functions will be based on the HYPRES database (Nemes et al. 2001). Subsequently, two different time-step normalisations and two different derivations of DegT50matrix values for each trial site will be conducted based on the recommendations of FOCUS (2006) and EFSA (2014). In the first case the estimation will be based on provided climate data by NASA and in the second case on JRC data. Finally, the degradation rates derived for each site either by using NASA or JRC data will be compared with the degradation rate based on measured soil moisture and soil temperature. The aim is to identify the best climate data provider (NASA vs. MARS), i.e. the one providing data source resulting in DegT50matrix values for the six trial sites the closest to the real DegT50matrix values.

MO101

Distribution of current use pesticides and personal care products in surface marine sediments from Vigo Estuary and Mar Menor Lagoon

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Coastal areas are exposed to high anthropogenic pressures (industrial activities, agricultural growing, urban sewages, harbours, uncontrolled spills…).Consequently many organic compounds enter to the environment and to the sea, which is the last recipient of significant part of these pollutants. Depending on the predominant activities in every coastal area many different contaminants can be found, but only a reduced group of them are currently monitored. In order to improve future monitoring programs it is necessary to identify which contaminants can be affecting to coastal areas and should be included in those programs. A pilot study to characterize the presence and distribution of organic contaminants of emerging concern in surface sediments from Vigo Ria and Mar Menor lagoon has being developed through IMPACTA project. Previous information is available about the distribution of PAHs and organochlorinated compounds in those areas but no information is available about the occurrence of current used pesticides and personal care products. In this study the distribution of current used pesticides (organophosphorus, triazines and others) and personal care products in surface sediments was characterized in spring and autumn of 2015. Pesticides were extracted from sediments using ultrasonic extraction and were analyzed by GC-MS. Herbicides (terbuthylazine, propyzamide, etc), insecticides (chlorpyrifos, methyl-chlorpyrifos, etc), personal care products (galaxolide, triclosan, etc) and other pollutants (tributyl-acetyl-citrate, tributylphosphate, etc) were found in surface sediments in the low range of ng g^{-1}. More pesticides were found in sediments sampled from Mar Menor than Vigo Ria ones due to the intensive agricultural activity in that area. However, the higher concentrations of plasticisers were found in Vigo Ria. The distribution of all the contaminant groups studied was heterogeneous, and significant seasonally differences were observed in the concentrations of organic pollutants. The higher concentrations were detected in depositional areas and the influence area of main urban nuclei, ports and agriculture areas.

MO102

Step4ward - An Efate toolbox

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'Step4ward' is a user-friendly Windows® toolbox to automate PEC_{sw} calculations, extract data from the models TOXSWA and SWAN and create MS Word® tables in the current dRR format. Software tools are very helpful and well established for modelling environmental fate and exposure of plant protection products and also for decision making in a regulatory context. Although the models were developed for regulatory purposes, model outputs, mostly text files, are not ready for use in regulatory reports or dossiers but need further formatting. Remarkably, the EU provides dRR templates with ready-made tables for predicted environmental concentrations in soil and surface water which are harmonised with the ecotoxicological section. Therefore there is a high potential for reducing report and dossiers workload for all involved parties. The 'Step4ward' toolbox was developed in the modern programming language VB.Net and is covering three main tasks: 1) automation of PEC_{SW} calculations at Step4, 2) extraction of TOXSWA and SWAN data from text files and 3) creation of appropriate Word tables in dRR format. Besides the availability of a stable ECPA tool (SWAN 4) for running PEC_{SW} at Step 4 'Setp4ward' includes advanced batch functionalities. These can be used to create and run SWAN input files automatically, considering standard requirements from current surface water guidelines (FOCUS 2007, 2015) like FOCUS runoff reduction factors for 10 and 20 m vegetated buffer strips or dry deposition values from the German exposure model EVA. Moreover, it is possible to link the batch functionality with an ecotoxicological RAC value to allow for performance of relevant runs only. The collection of all relevant data for the risk assessment (like maximum PECs, time weighted average PECs, main entry routes, selected application dates by PAT etc.) from TOXSWA output and SWAN input files is a time consuming process. 'Step4ward' provides a routine for that purpose and collects the information in temporary VB.Net data tables. From here, several routines can be run e.g.creation of an Excel overview of data or export of data to dRR Word tables. At present main functionalities for writing reports and dossiers are already available within 'Setp4ward' and a variety of more output options can be added to the toolbox in the future.

MO103
Evaluation of pesticide trapping efficiency equations for vegetative filter strips (VFS) using additional experimental data
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Vegetative filter strips (VFS) are widely used for mitigating pesticide inputs into surface waters via surface runoff and erosion. To simulate the effectiveness of VFS in reducing surface runoff volumes, eroded sediment loads and pesticide loads the model VFSMOD (Muñoz-Carpena and Parsons, 2011) is frequently used. While VFSMOD simulates infiltration and sedimentation mechanistically, the reduction in pesticide load in surface runoff by the VFS is calculated with the empirical multiple regression equation of Sabbagh et al. (2009). This equation uses the following inputs: predicted reduction of surface runoff volume and eroded sediment load (deltaQ and deltaE, respectively), absolute surface runoff volume and eroded sediment load entering the VFS, linear adsorption coefficient Kd of the pesticide, and the clay content of the field soil (as a proxy for the clay content of the eroded sediment). The regression equation of Sabbagh et al. (2009) has not been widely accepted by regulatory authorities, because its reliability has not been sufficiently established yet. Bach et al. (2016) concluded that the equation does not seem to be fundamentally flawed, but that evaluation against additional experimental data is necessary. In particular, the equation needs to be tested against cases with low deltaQ and high deltaE, which were not present in the calibration and validation data sets used by Sabbagh et al. (2009), but are predicted frequently by VFSMOD. Recently Chen et al. (2016) proposed an alternative regression equation, derived from the same experimental data as used by Sabbagh et al. (2009). The objective of the present study was to improve the validation status of the Sabbagh et al. equation by testing it against additional validation data. For this aim, a number of experimental VFS datasets not used by Sabbagh et al. (2009) were compiled from the available literature and checked for their suitability for testing. Many datasets had to be discarded in this process because suspended sediment loads in surface runoff had not been measured. Both the equations of Sabbagh et al. (2009) and Chen et al. (2016) were tested against the experimental datasets that were found suitable. The poster will present and discuss the main findings of the evaluation study.

MO104
A case-study of pesticide risk assessment to aquatic biodiversity of kettle holes in Northeast Germany using SYNOPS-WEB (with integrated PRZM)
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The moraine landscape of Northeast German lowlands is typically characterised by a dense distribution of kettle holes resulting from the last glaciations. Kettle holes are usually small, shallow, pond-like depressions with a dry-wet cycle. Often located in arable fields, they are hotspots of biogeochemical processes and biodiversity. Though protected by law, pesticide wash-off and soil erosion associated to intensive land-use impair their ecosystems functions and structural stability among other factors. We use the newly improved environmental risk assessment tool SYNOPS-WEB, integrated with the run-off model PRZM, to estimate risk from pesticide use to species relevant for aquatic biodiversity in four select kettle holes. We also comparatively analyse the predicted environmental concentrations (PECs) from SYNOPS-WEB with data obtained from the chemical monitoring of these kettle holes. The data on pesticide concentrations are part of an extensive pesticide monitoring program in the Uckermark region, about 90 km north of the Berlin, Germany. All four kettle holes, located in the same arable field, were monitored for pesticides seven times in 2016. Five pesticide applications, with 12 active ingredients in total, were carried out on the field in the same year. SYNOPS core algorithms estimate PECs via drift, runoff, erosion and drainage to the kettle holes. PECs are compared against measured concentration using Lin's concordance coefficient. Acute and chronic risk to aquatic biodiversity is then calculated for five reference organisms (*Daphnia* sp., *Chironomus* sp., aquatic plants (*Lemna* sp.), algae and fish) as Exposure-Toxicity-Ratios (ETR). Structural damage to the kettle holes in the form of soil erosion is also quantified as soil loss from the fields. We show that predictions of SYNOPS-WEB with integrated PRZM have the potential to depict pesticide contamination of small agricultural water bodies. SYNOPS-WEB risk assessments further revealed risks to agricultural water bodies that were not monitored for pesticide contamination. However, deviations of modeled results from monitoring data also reveal impacts of transport processes related to long-range transport.

MO105
H2Ot-Spot Manager NRW, a web based tool to assess the risk on aquatic organisms and the impact of mitigation measures
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This poster introduces the H_2Ot-Spot Manager as a web-based analysis and decision support tool to assist farmers and advisors in selecting appropriate mitigation measures or reduced-risk pesticides. The initiation of comparative assessment at user level is one of the subjects of EU 2009/128/EG (EuropeanCommission, 2009) and subsequently is an element in the national action plan. On behalf of the Ministry for Climate Protection, Environment, Agriculture, Nature Conservation and Consumer Protection of the State of North-Rhine Westphalia (MKULNV), the tool was developed and tested. It is based on the risk indicator SYNOPS in combination with models used in pesticide registration to assess runoff (PRZM) and filter strip function (VFSMOD). It allows farmers and advisors to conduct a realistic risk assessment of pesticide applications under specific environmental field conditions using data bases specific for NRW. Extended geodatasets on land use (InVeKos), surface water (GSK3C), slope (DGM10), soil properties (BK50) and grid based weather data (DWD) are linked to the tool and support the user to select the specific field and field conditions. The pesticide applications are entered by the user, assisted with entry suggestions from the German database for registered products (BVL). After a status quo calculation, possible mitigation measures which are relevant for the region can be selected from a catalogues. The SYNOPS core algorithms estimate PECs via drift, runoff, erosion and drainage to surface water. Acute and chronic risk to aquatic biodiversity are then calculated for five reference organisms (daphnia, fish, algae, lemna, and chironomus) as Exposure-Toxicity-Ratios (ETR). In common practice pesticide risk is not a determinant for farmers' decisions on its pesticide use. To change this, we believe that online tools such as the H_2Ot Spot-Manager with user friendly interfaces are inevitable instruments for farmers, advisors and authorities to support IPM strategies.

MO106
Risk Assessment for Protected Crops - Approaches Towards Harmonized Leaching Scenarios - GASP-S v 1.1
S. Sittig, Dr Knoell Consult GmbH / EFate Modelling; J. Witt, Bayer CropScience
Groundwater assessment in protected crops is driven by several factors, such as temperature, evapotranspiration, and irrigation. Irrigation scheduling plays an important role in greenhouse cultivation. The recently published EFSA guidance on protected crops (EFSA, 2014) provides recommendations on exposure assessment in protected crops in the regulatory context. However, only an illustrative, non-representative example scenario is provided for ground water exposure assessment in walk-in tunnels and greenhouses. Applicants are required to develop suitable scenarios. We present a scenario for standard walk-in tunnels and low-tech greenhouses in line with EFSA guidance on protected crops and with a coherent consideration of the driving factors, such as irrigation and evapotranspiration. The proposed methodology for evapotranspiration and irrigation produces a scenario that matches the criteria as laid down in the ESFA guidance and meets the validation criteria (%-tage recharge; optimal wetting conditions; total irrigation amount). The developed scenario provides a suitable basis for leaching assessment in Southern European greenhouses and walk-in tunnels. Hence, the framework constitutes a further development of the previously presented Groundwater Assessment Scenarios for Protected Crops in Southern Europe (GASP-S; Version 1.0) in terms of an enhanced representation of management practices in irrigation and off-season procedures. Scenarios for the calculation using PEARL 4.4.4 are made available.

MO107
A generic modeling of transport of plant protection product metabolites in the subsurface by coupling PEARL and OpenGeoSys
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The geometric characteristics of fields treated with plant protection products (PPP) can influence the measured concentrations of the active substance(s) and/or metabolites at downstream groundwater monitoring wells. In the current study, a generic modeling exercise was conducted to investigate the influences of shape, location and size of fields treated with PPP on the measured concentration at monitoring wells, thus to identify important factors influencing the representativity of measured concentrations with respect to the treated upstream area. Two scenarios with different configurations of treated fields were simulated by coupling the leaching model FOCUS-PEARL with the finite element simulation software OpenGeoSys. Leaching simulations for the fields were carried out using FOCUS-PEARL Hamburg scenario. The concentrations of PPP metabolites and the volume fluxes of water calculated in the leaching simulations were used as the

upper boundary conditions for the subsequent two-dimensional groundwater flow and solute transport simulations carried out using OpenGeoSys, in which resulting concentrations at a downstream monitoring well were calculated. The simulation results showed that the total area of treated fields upstream of the well is not necessarily the determining factor for measured concentrations at monitoring wells. Rather the coverage of the upstream flow path where there is a hydraulic connection to the monitoring well is the key factor influencing measured concentrations at the well. If the coverage of treated fields along an axis which is connected to the well and aligned with the groundwater flow direction reaches a certain threshold length, a further increase of the treated areas along that axis will not lead to higher measured concentrations at the well. The threshold length for a given well will be different from case to case, depending on factors such as hydraulic gradient, groundwater flow velocity and annual groundwater recharge at the site.

MO108

Time of Flight Modelling in Support of Monitoring Studies

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Dedicated edge-of-field modelling studies require knowledge of transit times from time of application to arrival at the groundwater surface in order to know the potential length of any proposed monitoring study. We term this time the “time of flight” (ToF). This information can also be useful in support of monitoring studies in order to demonstrate that the study was conducted for long enough to demonstrate the chance of exposure. We show that by extending standard FOCUS profiles, and by using the FOCUS PEARL model, realistic estimates of the ToF for various substances can be made. We demonstrate this by comparison with data from a variety of sources such as lysimeters, Prospective Groundwater Studies (PGWs) and dedicated edge-of-field monitoring studies. This approach has proved useful establishing several monitoring studies and has also been used to quantify how far the site history of applications needs to be made in order to be relevant to the proposed monitoring study. The estimates produced by this approach are approximate but useful. We show that estimates of time-of-flight that are realistic but that concentrations predicted by this approach are not relevant due to the large number of assumptions that have to be made.

MO109

Are agricultural soils "contaminated sites" from the viewpoint of residues of currently used pesticides and release of them to water?

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In surface and ground water monitoring, currently used pesticides (CUPs) or/and their transformation products have been repeatedly found at high frequency and at considerable concentrations. Are agricultural soils in watershed a secondary source of CUPs in ground/surface, because they keep CUPs residues for long time and slowly release them to water? Surprisingly, there are no data available for CUPs in soils in EU to answer this question. Not only for the purpose above, but also for the post-registration control of approved plant protection products, the monitoring of CUPs in Czech agricultural soils was performed in 2015. In 75 soils, 72 active ingredients and 24 transformation products were determined by multiple residue method using QuEChERS extraction and UPLC-MS/MS detection. The results identified several CUPs frequently found above LOQ: the most frequently azole fungicides epoxiconazole (36% of samples), tebuconazole (27%), flusilazole (17%), prochloraz (16%), and propiconazole (10%). Their mean concentrations in positive samples were about 0.01 mg/kg (i.e. Czech limit for pesticides in agricultural soils). The second most frequently detected compound was 2-hydroxy atrazine (29%) with mean concentration in positive samples about 0.03 mg/kg and the highest measured concentration of 0.135 mg/kg. The soil residues of CUPs should be definitely considered and soil monitoring programs for CUPs should be initiated in EU countries along with water monitoring.

MO110

Assessing surface water vulnerability to pesticides from monitoring data: a territorial approach

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In the last decades, several monitoring programs were established as an effect of the EU Directives that included requirements for monitoring the quality of water resources (drinking water, groundwater and surface water). Plant Protection Products (PPPs) are an obvious target for monitoring activities, since they are directly released into the environment. With the came into force of the Sustainable Use of Pesticides EU directive monitoring activities became even more important as a tool to identify water systems at risk and consequently to establish on a territorial scale areas where to implement risk mitigation measures. For instance, in the Italian National Action Plan, monitoring data of PPPs in surface water systems have been identified as the key indicators to achieve the sustainable use of PPPs on the territory. However, monitoring of pesticides is a challenging task because of the high number of registered pesticides, cost of analyses, and the need for sampling to be performed during periods of application and use, and under various weather conditions. Extensive data sets of high quality are consequently often missing. More in general, the information that can be obtained from monitoring studies are frequently undervalued by risk managers. In this study, we propose a new methodology to assess the degree of vulnerability of surface water to PPPs on a spatial context making use of monitoring data, providing indications about the need to implement mitigation measures in selected areas. The methodology evaluates the active ingredient impact through two distinct phases: acquisition of monitoring data and creation of informative layers of georeferenced data (phase 1) and statistical and expert analysis for the identification of areas at risk (phase 2). In particular, source data for the evaluation are geo-statistical elaborations of monitoring data in comparison with Environmental Quality Standards and Predicted No Effect Concentrations. Two different algorithms identify water bodies potentially vulnerable to the monitored substances, and narrows the selection to those areas where implementation of limitation or mitigation measures are suggested, taking into account the trend of contamination and the risk level in each selected monitoring station. This approach has been applied as a case study at the whole territory of Lombardy Region (Northern of Italy) for the substance glyphosate, proving useful considerations for regional water quality managers.

MO111

Modelling soil exposure to pesticides at territorial level

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Plant protection products (PPPs) are extensively used in intensive agriculture and they could reach non-target organisms following different paths; since the first regulatory acts, the European Community paid attention mainly to surface and groundwater media, where PPPs could reach humans through drinkable waters. Only in recent years, persistence of PPPs in soil has been considered in the context of approval at the European Union (EU) level with a former guideline document from DG-SANCO and the EFSA document “Guidance for predicting environmental concentrations in soil” in 2015. The latest guidance document states that the recommended exposure assessment consists of five tiers, using iteratively simple analytical models or numerical models for predefined or crop- and substance-specific scenarios; in particular, tier 4 consists on the application of spatially distributed modelling with numerical models (adapted versions of PELMO and PEARL model) using a fixed set of raster maps of environmental variables at EU level and clustering the resulting scenarios in order to strongly reduce the number of simulations. In this study, we propose a different spatial schematisation making use of a web-GIS expert system (VULPES) already developed for groundwater exposure assessment at territorial level. VULPES has been adapted to soil exposure assessment calculating the 90th percentile concentration in total soil averaged over the top 5 and 20 cm of soil. It implements the latest FOCUS version of PELMO and PEARL models, simulating all the unique combination of weather, soil and irrigation areas identified by the intersection of detailed vectorial maps of soil pedology, weather parameters and irrigation scheduling. Results are then collected in a soil vulnerability maps and the spatial 95th percentile of the PEC in soil within the region is calculated directly on resulting data from simulations. VULPES could be used by risk managers to investigate the impact of PPPs on regional soils and to verify the appropriateness of the current monitoring network or to suggest its repositioning. A test case has been deployed in Lombardy region (Northern of Italy).

MO112

Effects of (nano)formulations on the fate of bifenthrin in soil and consequences for exposure assessment

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The study aimed at evaluating (i) the extent to which formulations may affect the fate of an insecticide in soil, and (ii) how discrepancies could be integrated in fate modelling for environmental exposure assessment. Sorption and degradation parameters of bifenthrin were measured for (i) the pure active ingredient, (ii) a series of nanoformulations, and (iii) a commercially available formulation. In most cases, fate parameters derived for the formulated insecticide were significantly different to those derived for the pure active ingredient. The processes behind the discrepancies remained difficult to isolate, mainly due to the absence of adequate analytical strategies to detect and characterize formulated pesticides in environmental matrices. Finally, experimental fate parameters were used to carry out a series of exposure modelling exercises. Predicted environmental concentration very much depended on the modelling approach adopted to account for the impact of formulations on fate parameters. Overall, this study represents the first step towards the development of suitable approaches to evaluate the fate of formulated active substances. Such approaches are needed to support the design of more intelligent (e.g. nano-enabled) delivery systems for pesticides and other bioactive substances, while ensuring their robust assessment by regulators. Kah et al. (2016) *ES&T.* 50, 10960-10967.

MO113
The effect of freezing and thawing on the transport of pesticides in soil
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There is a great focus on the use of pesticides in general but also more specifically on the contamination of groundwater and surface water. Local climatic conditions can influence the fate and behaviour of pesticides to great extent. Not many studies have investigated experimentally in the laboratory the effect of freezing and thawing and the preferential transport of pesticides in soil. Studies with tracers or field measurements indicate however that solutes and pesticides can leach to groundwater or surface water at relative high concentrations during snowmelt and freezing and thawing episodes during winter and early spring. A leaching experiment with undisturbed soil columns from topsoil and subsoil from two different agricultural soils (silt and loam) was performed to study water flow and pesticide transport in frozen soils under cold climate conditions. The aim was to see whether the transport of pesticides in frozen soil was significantly different from transport in unfrozen soil. The results from the top 20 cm of the loam soil will be presented. The results from the study clearly indicate differences between frozen and unfrozen soil and consistently show that both the inactive tracer bromide and the pesticide MCPA are transported preferentially through macropores in the frozen soil resulting in high concentrations in the leachate. Hence, indicating the potential transport of autumn applied pesticides from soil to water during winter and spring under cold climate conditions. The results also show a difference between the bromide tracer and the mobile pesticide MCPA, especially in unfrozen soil, with relative concentrations and total amounts of MCPA leached being significantly lower than for bromide.\n

MO114
Evolution of ambient air concentrations of 46 pesticides in Wallonia, Belgium in summer 2015
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The Directive 2009/128 of the European Commission establishes a framework to achieve a sustainable use of pesticides by reducing the risks and impacts of their uses on human health and the environment. Each Member State has to develop a national plan that fulfils the directive's requirements. In Belgium, the national plan is composed of federal and regional plans that present specific and joined actions. In Wallonia, one of the objectives of the Walloon Plan for the Reduction of Pesticides (WPRP) is to create information collection systems of chronic poisoning more specifically targeting any groups which could potentially regularly face pesticide exposure, such as users, farmworkers, or people living near to areas where pesticides are applied. Human can be exposed to pesticides through 3 main pathways, namely dermal exposure, ingestion and inhalation it is important to assess the environmental concentrations. Even though ingestion is considered as the main exposure pathway it is now suggested that inhalation might also be important in rural but also in urban locations. Indeed studies in North America and Europe have reported air concentrations in rural and urban locations as high as several hundreds of ng/m^3. Therefore, assessment of CUPs concentrations in ambient air is important for better understanding of non-dietary human exposure and potential health effects. As compared to water and soils CUPs concentrations were never measured in ambient air in Belgium. Therefore as requested in the WPRP information on ambient air concentrations have to be collected. In this study, ambient air concentrations of 46 currently-used pesticides were assessed in Wallonia between 25th June and 20th August 2015. Pesticides analysed were chosen using different criteria (*i.e.* probability of presence in ambient air, uses in Wallonia, chronic toxicity and methods of detection) through a prioritised list. In order to assess spatial variations of air concentrations and therefore to provide a better insight of the population exposure in Wallonia, different typologies were defined for the 12 sampling sites: 2 reference sites (low or no uses of CUPs), 2 urban sites, 5 agricultural sites and 2 non-agricultural use of CUPs sites. To assess temporal variations ambient air was sampled for 14 days with an active air sampler. Results of CUPs concentrations in ambient air will be presented. Moreover, spatial and temporal variations will be discussed.

MO115
Pesticide field fate studies to elucidate and quantify important sources and loss pathways
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The use of generic default values in current regulatory modelling frameworks describing pesticide loss pathways and possible exposure mitigations , especially at the field scale, may result in difficulties in demonstrating regulatory compliance for specific pesticide uses and compounds that do not fit the standardised exposure assessment approach. In these cases, higher tier field studies may be a preferred option to demonstrate safety directly through environmental monitoring or generate use or compound specific model parameter or mitigation effectiveness values. However, the current lack of guidelines available for the conduct of many such studies has meant that acceptability of the experimentally derived data might be variable across the regulatory processes and EU Member States. This poster describes experiences in designing and carrying out a range of higher tier field studies for a variety of exposure sources and pathways aimed at providing application/use or compound specific data that should be considered suitable to be used directly in the risk assessment process in place of generic model parameter or effectiveness values or even simulated exposure. The scope for providing suitable data for this purpose is discussed, along with available and emerging guidelines, as well as acceptability issues, for example concerns regarding replication and data validation.

MO116
Simultaneous air and water passive sampler modelling with ethylene-vinyl acetate: theory and experiment
J.M. Parnis, Trent University / Chemistry; A. Abdollahi, Trent University / Department of Chemistry; A. Celsie, Queens University; T. Harner, Environment Canada / Atmospheric Science Technology Directorate; D. Mackay, Trent University / Chemistry
We apply a multimedial fugacity-based modelling framework to the simultaneous uptake of PAHs from equilibrating water and air to EVA-coated bead samplers. COSMO-RS theory is used with a liquid oligomer approach to EVA modelling to estimate EVA-air and EVA-water partition ratios. Together with experimentally obtained data for concentration changes in water, air and samplers over a 200 hour period, a complete picture of the dynamics of the system will be described. The results allow us to determine mass-transfer coefficients for use in other estimations of time to equilibrium as a function of EVA film thickness and deployment medium, as well as chemical-specific parameterisation of EVA as a passive sampling medium.

MO117
Export Crops and MRLs: Implications for Ecological Health in Low-Regulation Environments
C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering; A. Mendez, ETH Zurich / Safety and Environmental Technology Group
Many economically important fruit crops are grown in tropical environments in regions where there are relatively few or lax environmental regulations when compared to North America or Europe. However, these crops are required to meet maximum residue limits (MRLs) in order to be exported and sold in these regions. Because of this, MRLs often constitute de facto regulations in the growing environment that are set by the importing countries. Although this has led to the phase-out of a number of persistent and toxic pesticides in these tropical regions, it has also led to use of less persistent pesticides that nevertheless have very high aquatic toxicity. Therefore, even when fruits like bananas and pineapples consistently meet export requirements, the local growing environment may experience short-term peaks in aquatic concentrations of pesticides following runoff events that result in large fish kills. Here, we discuss dynamic modeling approaches to understanding the time- and application-dependent environmental pesticide levels that can result given the MRLs for current-use pesticides in fruit crops. We present the particular example of banana cultivation in Costa Rica and show how different application patterns, pesticide physicochemical properties and local environmental conditions interact to determine the ecological implications of MRLs defined solely to protect human health.

MO118
Sorption and leaching behaviours of phenylurea herbicides in tropical soils: implication for modelling
B.K. Agbaogun, University of Ibadan / Chemistry; B.I. Olu-Owolabi, University of Ibadan / Department of Chemistry; K. Fischer, University of Trier / Department of Analytical and Ecological Chemistry
The sorption and leaching behaviours of two PhenylUrea Herbicides (PUHs), Diuron and Linuron were studied in ten tropical soils (majorly of Alfisol, Entisol and Inceptisol orders on the USDA soil taxonomy) from the Southwestern Nigeria. As the goal of this work was to determine the soil specific parameters that are most responsible for sorption, and by extension leaching, extensive efforts were made to characterize the soils, using standard methods. Sorption isotherms were obtained by equilibrating the soil samples with 0.001molL^{-1} $CaCl_2$ solutions spiked with increasing concentration of the target PUH in the range (0.5- 25gL^{-1}). HPLC-DAD at 250nm was used to quantify the equilibrium concentration after 24 hours contact time. The linear Freundlich isotherm equation was appropriate to describe the sorption behaviours of both compounds in the soils ($R^2 \geq 0.96$ for Diuron, and $R^2 \geq 0.98$ for Linuron), and nonlinear adsorption isotherms ($1/n < 1$) were observed in all cases. Also, the mean of value of single-point distribution coefficients (K_d) at seven concentration levels was calculated for each soil. K_f values ranged from 5.4-18.9 and 6.0-29.2 mLg^{-1} for Diuron and Linuron respectively, while Kd values ranged from 4.0-16.0 and 5.0-29.2 mLg^{-1} respectively, for Diuron and Linuron. Both K_d and K_f show high correlations with soil f_{oc}, and K_{oc} values were

found to range from 365-809mLg and 513-809mLg respectively, for Diuron and Linuron. This indicates that sorption of the two PUHs in the soils was more of hydrophobic interaction between the PUHs carbon chain and the soil organic matter. The K_f and K_d also follow the sequence Linuron > Diuron, which is the same order for their K_{ow}. This suggests that not only the soil characteristics (basically the organic matter content), but also the physicochemical properties of the PUHs influence their sorption pattern in soils. The Gustafson Ubiquity Score (GUS) index was calculated for each compound based on the average $t_{1/2}$ values reported in the literature, and the K_{oc} reported in this work. Both compounds were found to show moderate to slightly high leaching potentials in the soils (2.04-2.9). Further work is ongoing to obtain advanced physical and chemical data of the soils for better interpretation of the chemical processes. The results in this study should assist in the modeling of pesticides fate and in the risk assessment for tropical soils which are the main objectives of this work.

MO119

Integrated risk assessment of pesticide, the use of MERLIN-Expo tool for combining exposure assessment with toxicological effects on the food web

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Recent developments in the field of Environmental Risk Assessment (ERA) recommend the need for a better integration between the exposure assessment and the toxicological effects. The scope of the present work is to investigate the possibilities given by MERLIN-Expo Tool, a recently developed exposure model, to estimate the pesticide environmental exposure and to simulate its accumulation in the aquatic food web through the trophic levels. MERLIN-Expo was developed in the frame of the EU project 4FUN in order to provide an integrated tool for the exposure assessment of the environment, biota and humans to metals and organic chemicals, including pesticides. Indeed, within the project, the SWOT analysis of the tool highlighted its possible employment in several European legislative frameworks. The European authorisation process of plant protection products requires to address the potential for bioaccumulation and biomagnification via the food web and its effects to birds and mammals, which is a critical issue for persistent compound. However, the current evaluation is based, at lower tier, on highly generic assumptions and equations, and no harmonised approaches are available for higher tier assessments. We simulated a typical use pattern of a pesticide sprayed to orchards according to agricultural practices in southern Europe. For scenario development the field and the water body parameterization, as well as the meteorological dataset, were taken from SW FOCUS recommendations. A generic food web for the water body considered in the pesticides fate assessment scenarios, was created keeping into account the outline provided by the EFSA guidelines. We identified advantages and limitations in the use of MERLIN-Expo, including the possibility, given by the model, to perform probabilistic simulations. Uncertainty and sensitivity analysis was conducted in order to investigate the impact of uncertainty and variability associated to input parameters on the final outputs. Finally, the possibility of using MERLIN-Expo for regulatory purposes in the EU pesticide registration according to Reg. (EC) 1107/2009 was evaluated.

MO120

Good agricultural practice (GAP) in the light of meteorological constraints

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When applying pesticide sprays, the principles of good agricultural practice (GAP) have to be taken into account. For instance, pesticide sprays should not be applied when the weather conditions are not favourable for an effective and efficient use of the chemicals. Although this seems reasonable and clear, in practice it can offer various difficult situations for farmers where they have to decide whether or not to postpone a spray event or other treatment. This paper deals with the statistics of meteorological circumstances and probability aspects of carrying out agricultural treatments conform GAP. Weather conditions on an hourly base for several decades are available from different weather stations in the Netherlands. Probabilities of unfavourable weather conditions are computed and discussed. Such conditions may involve situations with too high wind speeds or rainy conditions. The duration of such conditions is discussed as well. Correlations between for instance wind speed, wind direction, air temperature are investigated. Using the results from these investigations, the current study tries to identify and quantify the meteorological constraints while practicing GAP. Risk assessment models should be aware of these constraints and deal with it properly.

MO121

Modelling the fate of pesticides in European and French cropping systems: Integration of complex agricultural practices in the regulatory risk assessment

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Modelling the fate of pesticides in the environment can help prevent and manage soil and water contamination. In particular, groundwater protection is a key issue for human health and resources sustainability. At the European level, a pesticide shall only be approved if the predicted concentrations in groundwater (PECgw) of the pesticide or of relevant metabolites are below 0.1 µg/L. The PECgw have to be estimated using at least two of four recommended models (MACRO, PELMO, PEARL, PRZM) together with representative European agro-pedo-climatic scenarios available for each crop (FOCUS scenarios). These scenarios assume that the same crop is grown every year. This assumption can be considered as a worst case for PECgw assessment but it does not include crop rotation. The PECgw have to be lower than 0.1 µg/L for at least one of the scenarios for pesticide approval. At the French level, there are two levels of risk assessment for plant protection products (PPP) containing the approved pesticide(s). First, PECgw are estimated from the FOCUS scenarios. The PECgw have to be lower than 0.1 µg/L for all representative scenarios. If not, a refined level of assessment is required. One refinement is based on numerous French agro-pedo-climatic scenarios (FROGS scenarios) coupled with the PEARL model. In contrast to FOCUS scenarios, FROGS scenarios allow to define risk mitigation measures based on specific cropping practices (i.e crop rotation). In order to be as representative as possible of standard agricultural practices, typical crop rotations of 31 French agricultural regions are implemented in FROGS. PPP can only be approved for conditions where PECgw are lower than 0.1 µg/L. However, while these scenarios consider more realistic cropping systems than the FOCUS scenarios, they do not take into account agricultural practices such as cover crops, mulch… In addition, the representation of agricultural practices in the numerical models remains incomplete. Therefore, new tools are needed to consider the variability of cropping systems in risk assessment. Among the most recent developments, the combined use of a pesticide fate model, such as MACRO, and a crop model, such as STICS able to simulate the growth of a large range of crops under different agricultural practices (mulch, tillage...), is a promising method to refine risk assessment of pesticides. In the future, it would allow to consider more realistic cropping systems for pesticide regulatory risk assessment.

MO122

New software development for automated environmental fate modeling and reporting

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For the registration of plant protection products the agrochemical industry needs to provide environmental risk assessments for active substances and related products. To conduct these risk assessments, obligatory and officially provided third-party computer models are used in many countries worldwide. The computer models are used to obtain an estimate of the amount of substance that will enter the environment (Predicted Environmental Concentrations, PEC). PECs are calculated for different environmental compartments by different simulation models. Hence, in an environmental risk assessment several different models need to be set-up, parametrized, or run separately. This decentralized working procedure unfortunately implies significant drawbacks. The main disadvantages are: replicated entry of identical input data, great extent of manual work, high quality assurance effort, challenging data management due to scattered information of the input/output files, and time-consuming workflow documentation. As a consequence, we started to develop a software package to facilitate automated environmental exposure modeling and reporting. The aim was to create a software application that can drive all necessary computer models and evaluate the results from one single platform in an automated manner. The software is developed in a modular structure allowing for individual integration of modeling tools. This structure enables flexible adaptation at the modular-level, e.g. when new regulatory models are released, or new model versions are made available and need to be applied. The software suite will be provided as a web-based solution accessible through a common web browser. Model calculations will no longer need to be carried out on desktop computers, but will be performed on a more powerful and remote server located in a secured data center. The software will be able to automate and optimize the workflow for individuals or groups of people dealing with environmental fate and exposure assessment. It is developed to automate model parameterization and model simulations, extract the results and transfer the output to formatted tables suitable for dossier/report incorporation. The development is of interest, not only in terms of speeding up modeling and report generation, but also it will optimize the organization of data, reduce the occurrence of manual input errors and reduce the effort required for quality control.

Nanomaterial fate and toxicity - Implications of the environment as a global reactor for nanomaterials along their life-cycle (P)

MO123

Detection of CeO2 (nano) Particles in Sewage Sludge

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The goal of the study was to identify and quantify engineered nanoparticles (ENPs) in sewage sludge, which is expected to be a significant sink for ENPs. The selection of the sewage sludge samples was based on a broad survey of the total elemental contents in sewage sludge from 64 Swiss wastewater treatment plants. Tailored colloidal extractions of the sewage sludge in combination with analytical electron microscopy were applied to identify and quantify anthropogenic (nano) CeO_2 particles in the sewage sludge. Concentrations of cerium (Ce) and lanthanum (La) were quantified in acid digested sludge samples using a quadrupole dynamic reaction cell ICP-MS (Agilent 7500cx, Basel, Switzerland). Colloidal extracts of selected sludge samples were centrifuged onto poly-L-Lysine functionalized TEM grids. Particles were investigated using a scanning transmission electron microscope (2700Cs, Hitachi, Japan) and elemental analyses of individual particles was performed with an energy dispersive x-ray analysis (EDX) system coupled to the microscope. The total Ce concentrations in the sewage sludges were mostly around a few tens of mg/kg dry weight (dw), but ranged up to 900 mg/kg dw. The concentrations of La were always lower than the Ce concentrations, but mostly on a comparable scale. Although the natural backround concentrations of both elements are very variable, the ratio of Ce:La reported from soils is mostly around 2:1. Engineered CeO_2-NP, however, do not contain significant amounts of La. Thus, a sewage sludge having high Ce contents and a high Ce:La ratio was selected for electron microscopy analysis to assess whether the sludge indeed contained anthropogenic CeO_2-NP. Due to their high density, CeO_2 particles appeared as very bright particles in high angular annular dark field images, which greatly facilitated their detection. The size of the CeO_2 particles ranged from ~ 50 – 200 nm. EDX analysis of selected CeO_2 particles revealed significant La concentrations in some of the CeO_2 particles, but also showed CeO_2 particles without La. These 'pure' CeO_2-NP point towards an anthropogenic source of CeO_2particles. Additional TEM analysis of individual CeO_2 – NP in combination with single particle ICP-MS analysis will be used to quantify the CeO_2-NP abundances and to distinguish between natural and anthropogenic (nano) CeO_2 particles in the sewage sludge.

MO124

Toxicity of silver nanoparticles on two Lemna sp.: effects on photosynthesis and growth

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The widespread application of silver nanoparticles (AgNPs) in commercial products has considerably increased concern regarding risks to the aquatic environment. Although the toxicological effects of AgNPs to several aquatic organisms have been reported in literature, little is currently known about the potential toxicity of AgNPs to aquatic macrophytes which have an essential role in ecosystems. The aim of this work was to investigate the effects of AgNPs on two *Lemna* species (*Lemna minor* and *Lemna gibba*) using morphological and physiologicalendpoins (frond number and dry weight based growth rate, photosynthetic pigment content and chlorophyll fluorescence). Both species were exposed to AgNPs for 7 days. Results showed that AgNP toxicity is endpoint and species dependent. Regarding growth, dry weight based growth rate was found to be more sensitive to *L. gibba* while frond number based growth rate was found to be more sensitive to *L. minor*. Alterations on the photosynthetic pigment content and chlorophyll fluorescence proved to be good and sensitive endpoints to evaluate the toxicity of AgNPs to both *Lemna* species. Dose-dependent inhibitory effects caused by AgNPs were observed at all endpoints studied, even though at high concentrations. *L. gibba* was the most sensitive species to AgNP exposure considering all the tested endpoints. The results of this study enabled not only to generate further knowledge about the toxicity of AgNPs to aquatic macrophytes but also to show the need of considering the sensitivities' differences between species when examining the risks of AgNP to the aquatic environment. **Keywords:** nanomaterial, aquatic plant, chlorophyll, Tween 20

MO125

Toxicity assessment of four phosphorus adsorbents used for lake restoration

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At present, because of human action is altering biogeochemical cycles, phosphorus (P) reserves, using for making fertilizers, are being depleted, while aquatic ecosystems suffer a P enrichment due to the translocation of this element, causing the eutrophication of them. Being aware that the control of the external load is the essential step to restore eutrophied systems, several chemical adsorbents have been proposed as a management tool. The use of magnetic microparticles (MPs) is an innovative and promising tool for P removal from aqueous solutions and many advantages have been identified. However, before using them in a b_whole-lake application", it is essential to assess the possible toxicological effects of them and other commonly adsorbents used on aquatic biota. For this reason, *laboratory tests were carried out* in this research*, following standardized protocols, with the aim to assess the short-term effects of commonly used adsorbents like Phoslock and CFH12, and* promising novel adsorbents such as *commercial magnetite and* commercial micronsized carbonyl iron particles *(HQ) on the growth of the alga Selenastrum capricornutum and immobilization of the cladoceran Daphnia magna.* The algal growth inhibition test procedure was performed following the SOP version 1.0. of the ISO 8692 during 48 hours. The adsorbents concentrations used were 0.01, 0.05, 0.1, 0.5, 1 g l^{-1}. Moreover, the toxicological test with *Daphnia magna* was carried out during 48 hours following OECD 202 guideline for acute immobilization tests with *Daphnia* sp. using 0.0154, 0.0385, 0.096, 0.24, 0.6, 1.5, 2.25, 3, 3.75 g l^{-1} of adsorbents. The growth inhibition algal test showed the following results: for magnetite and Phoslock no pattern of algal inhibition was observed. HQ MPs caused an EC50 of 1.08 g l^{-1} on *Selenastrum capricornutum* growth, an EC50 much higher than found in a similar experiment with *Chlorella* sp. The EC50 result for particles of CFH12 was 0.42 g l^{-1}. In the immobilization test with *Daphnia magna*, the EC50 obtained were very similar for all the adsorbents tested (Phoslock: 2.14; HQ: 1.86; Magnetite: 2.76; CFH12: 2.26 without being statistically different for HQ, Magnetite and CFH12. In all the cases the EC50 obtained for our test compounds are higher than those obtained for other particles, it is necessary to evaluate the concentration that we must add to the ecosystem, which will depend on the concentration of phosphorus in the lake.

MO126

Differential effects of two metal binding proteins on the dissolution of citrate-coated silver nanoparticles

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Silver nanoparticles (AgNPs) are widely used for their antimicrobial properties and represent about 30% of nanotechnology-based consumer products. However, the question remains raised concerning their toxicity since the silver released from the AgNPs could replace some physiological metals. For example, thiol groups play important roles in silver chelation, and those present in biomolecules could be therefore potential silver chelating-groups. Thus, we attempted to characterize the interaction between citrate coated silver nanoparticles (20 nm) and two thiol rich metalloproteins. Their interactions with the AgNPs were studied by various complementary approaches: transmission electron microscopy (TEM), dynamic light scattering (DLS), UV-vis and circular dichroism (CD) spectroscopies. Exchanges of the endogenous metals by ionic silver originating from AgNP dissolution were also quantified by asymmetrical flow field-flow fractionation linked to inductively coupled plasma mass spectrometry (AF4-ICP-MS). For the first time our results presented in this work highlight that the AgNPs behaviors differ according to the thiol groups involved and the structures of the proteins, which might potentially lead to varied physiological consequences in living organisms.

MO127

Development of a dynamic model (SWNano) to assess the fate and transport of engineered TiO2 nanoparticles in sewer networks

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TiO_2 ENPs are widely used for paint, sunscreen, cosmetics, and solar cell. Because more than 60% of TiO_2 ENPs is transported into the natural surface waters via domestic wastewater, its potential health impacts on aquatic environment are of serious concern. Before entering into the aquatic environment, however, ENPs pass through sewer ducts where ENPs are expected to experience substantial changes in its characteristic properties by the processes including hetero-aggregation and sorption onto organic matters and other surfaces. Although a few multimedia models were developed to assess the fate and transport of ENPs in the natural environment often with a particular focus on aquatic systems, no model exists yet which can assess the fate and transport in the sewer systems. This study proposes a model, SWNano (Sewer-Water nano), that quantitatively simulates the spatiotemporal changes in the size distribution and the concentration of TiO_2 ENPs in a sewer network of Seoul metropolitan city. Five sewer structures were selected based on the statistical analysis of sewer pipes' slope, length, diameter, and the roughness coefficient. The flow of waste water in the sewer system is analyzed using the 'XP-SWMM model' which is input to SWNano for calculating the advection flux and water depth in the sewer pipes. Five size classes (i.e., 20, 50, 100, 300, and 500 nm) are used to represent the size distribution of the TiO_2 ENPs. Important variables in the model include suspended particulate matters in the wastewater and their density and size distribution as these variables strongly influence the main fate and transport processes of the ENPs such as hetero-aggregation and settling. Homo-aggregation is assumed to be negligible. The simulations indicate that most of initially dispersed TiO_2 ENPs entering the sewer system is hetero-aggregated or sorbed onto other surfaces. In comparison with the material flow analysis results of a Gottschalk' study, the present study predicts that substantially reduced quantity of dispersed TiO_2 ENPs will enter into the connected waste treatment plant or the natural aquatic system if directly discharged without treatment. It follows from the results that the fate and transport of TiO_2 ENPs in the sewer systems should be taken into account for the exposure assessment of aquatic ecosystems as they can significantly alter the concentration and the size distribution of the ENPs prior to arriving at the natural aquatic systems.

MO128

Determining the effect of Cadmium/Tellurium Quantum Dots on Staphylococcus aureus by comparing bacterial growth curves and xCELLigence as a potential real-time indicator for nanoparticle toxicity

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Nanoparticles can be defined as an ultrafine unit with measurements in nanometres. These particles may occur naturally in the environment, be released unintentionally by industrial processes or engineered for consumer products. They have unique characteristics and are used in a variety of areas such as, medicine, engineering, catalysis and environmental remediation. As the global use for NPs increases the risk of environmental contamination increases. It may also affect bacterial species associated with the gut and environmental micro-organisms, it is therefore important to understand the mode of action of these nanoparticle, as microbes forms the basis of various food chains. The aim of this study was to determine the effect of cadmium/tellurium quantum dots (QDs) on *Staphylococcus aureus* by using real time xCELLigence and comparing these results to standard bacterial growth curves. Three functional groups attached to the surface of the QDs were selected namely a positive group (NH_3), negative group (COOH) and a neutral group (PEG). Varying concentration were made up with MiliQ water (5, 2.5, 1, 0.5, 0.1 mg/L). Cultures were incubated at 37 C for 24 hours in Mueller Hinton broth before use. Optical density was measured at 600 nm and bacterial cultures were prepared to 10? CFU. For the standard bacterial growth curves the culture were incubated in Mueller-Hinton broth and the growth followed spectrophotometrically for 24 hours. The xCELLigence results were compared to standard bacterial growth curves. Results obtained from the growth curves showed clear inhibitory effect associated with all the functional groups bound to the quantum dots, with NH_3 showed the most pronounced effect. The effect seems to be associated with dissociation of cadmium from the quantum dots. The xCELLigence assays are currently in progress with optimising of biofilm formation underway. **Keywords:** Cadmium/tellurium quantum dots, xCELLigence, *Staphylococcus aureus*

MO129

The toxicity assessment of engineered nanoparticles in algal bioassays

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Rapid growth of the nanotechnology industry and increasing mass production of engineered nanomaterials in consumer products both have increased heated debates and public discussions on this matter. Nanoparticles may be released into the environment throughout either of the life cycle stage: from the moment of their fabrication and production up to the disposal of the final product in which nanoparticles are contained. Thus, is needed to have an assessment of the risks of engineered nanomaterials to the environment. Our experiments were carried out on cultures of unicellular green alga *Chlorella vulgaris* Beijer being in the exponential-phase. Strains of the *Chlorella vulgaris* Beijer were cultivated at the 36º? in the 50% liquid Tamiya culture medium coupled with continuous stirring. Engineered nanoparticles of silver (Ag) with nominal particle size of 15-25 nm, at a concentration 250 ppm; of titanium dioxide IV (TiO_2) with nominal particle size of 100-190 nm and of silica nanoparticles (SiO_2) with nominal particle size of 10-15 nm and 100-120 nm were chosen as research objects. Samples were dissolved in distilled water and sonicated at a frequency of 35 kHz for 30 minutes. Toxicity assessment was performed by looking at the change of the relative index of chlorophyll delayed fluorescence (RIDF) and optical density (OD), according to the following methods. The toxicity criteria of engineered nanoparticles on the strains of algae was judged as inhibitory concentration EC_{50} which causes a 50% reduction of recorded parameter compared to the control sample. The results reveal that the greatest inhibitory effect on the culture of *Chlorella vulgaris* Beijer was caused by Ag nanoparticles – the EC_{50} values were 0.05 and 0.11 mg/L for OD and RIDF respectively. The comparison of the toxicity carried out between Ag nanoparticles and its ionic form shows that the ionic form is more toxic than nanoparticles by 2 orders of magnitude for OD and RIDF. It was found that TiO_2 nanoparticles reduce the rate of growth and photosynthetic cell function of algae insignificantly. It was conducted that the more SiO_2 nanoparticles size was the greater the toxic effect was. Therefore, the value of EC_{50} for OD with nanoparticle size of 100-120 nm was 8 mg/L. Moreover, the hyper-toxic effect has been registered using RIDF. However, the same experiment conducted on SiO_2 nanoparticles with particle size of 10-15 nm showed no toxic effect at all.

MO130

Iron oxide nanoparticles effects on Daphnia magna: Implications for environmental remediation

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Iron is an abundant and an essential nutrient in the environment, and naturally occurring nanoparticles containing iron are ubiquitous. Lately, synthesized iron oxide nanoparticles have been used in environmental remediation as an alternative to micro iron oxide. These particles have specific properties that make them more efficient; however these specific properties might be the cause of concerns over their behaviour and toxicity. In addition, nanoparticles usually have a surface functionalization which could be relevance for their toxicity and risk assessment. During nanoparticles exposure, initial conditions can rapidly change, hindering to establish the mechanisms of toxicity and making difficult to obtain reproducible results. In this work, we examine the aquatic effects of humic acid coated iron oxide nanoparticles (ha-IONP) in *Daphnia magna*. Humic acid and ha-IONP were found clogging up the filtering apparatus, and/or adhered to the exoskeleton, hindering the swimming and molting, and causing the immobilization and death of the organisms. Our study showed a low toxicity of these nanoparticles ($EC_{50} > 900$ mg/L), mainly related with the physical interaction of the humic acids, NP aggregates and a mixture of both with the daphnids. Although any hazard should be taken into account when using these nanoparticles in environmental remediation, these effects should be evaluated in comparison to potential benefits of detoxification of contaminated waters, and all should be balance together in remediation scenarios.

MO131

Effects of silver nanoparticles from effluents of wastewater treatment plants (WWTPs) on the behaviour of Daphnia magna.

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Over the last ten years the amount of manufactured nanomaterials (MNMs) has been increasing in the environment due to their use in products such textiles, cosmetics, sunscreens, and paints. MNMs accumulate in the aquatic ecosystem after passing wastewater treatment plants. The remaining MNM levels may cause increased toxicity for the aquatic environment. One of the most abundant MNMs are silver-nanoparticles (Ag-NPs) and, therefore, they represent a risk for the aquatic environment and the aquatic food web. To investigate the biological impact of Ag- MNMs and Ag-NPs from effluents from WWTP we focus on daphnia as a key species in a typical food web in a freshwater system, and a well-established model species for ecotoxicological and ecological studies. A change in daphnia behaviour triggered by Ag-NP exposure could be a sensitive endpoint for risk assessment. An innovative intelligent 3D-computer vision system has been established to automatically track and analyse the behaviour of *Daphnia magna* during exposure to Ag-MNMs and Ag-NPs from WWTP effluents under standardized conditions. Our aim is to understand whether and how Ag-NPs in pure forms (NM300K) and Ag-NPs from WWTP effluents affect the movement and the behaviour (swimming velocity, average speed, swimming height, turning based features and speed distribution) of *Daphnia magna*. We exposed 10-day old daphnia to different Ag-NPs concentrations and recorded the behaviour at eight recording times (0h, 3h, 6h, 12h, 24h, 48h, 72h, 96h). Our study was performed after the OECD guideline No. 202 with extended test duration (96h). At each recording time 10 daphnia (5 replicates) were exposed in 100 mL cuvettes and movement was recorded for 2 minutes. We aimed to make a statistical analysis of different indicators which might be connected to the behaviour of each individually tracked daphnia. These analyses can be used to evaluate statistically significant differences between exposed daphnia and control daphnia. A video with more information and a visualisation of the tracking system can be found at youtu.be/uYq KEY9RJ4.

MO132

Effects of Wastewater Borne Silver Nanoparticles on the Reproduction and Biochemical markers of Daphnia magna

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Silver nanoparticles (AgNPs) are widely used in a variety of products, e.g. textiles, bandages, and building facades, mainly due to their antimicrobial properties. Their increasing use leads to the transfer of AgNPs into wastewater treatment plants (WWTP) via industrial and urban sewage. During water treatment, silver nanomaterials remain in the sewage sludge to a great extent. Only a small amount of mostly transformed AgNPs is finally released into the environment. In this study we investigated the toxic effect of pristine and transformed AgNPs on the reproduction and biochemical markers of the freshwater cladoceran *Daphnia magna.* Four model WWTPs were conducted according to OECD Guideline 303A. The collected effluents were used to perform a chronic reproduction test with *Daphnia magna* according to OECD guideline 211. Animals were exposed to (i) effluent from model WWTPs previously contaminated with AgNPs, (ii) uncontaminated effluent, manually spiked with AgNPs and (iii) dilution water enriched with pristine AgNPs. In a further approach, 96 h old daphnids were exposed to the same treatments for 4 days, collected and analysed for changes regarding the biochemical markers superoxide dismutase, glutathione S-transferase, thiobarbituric acid reactive substances to assess lipid peroxidation, lactate dehydrogenase, catalase and acetylcholinesterase. No chronic effects were found in *D. magna* after exposure to effluents with transformed AgNPs. However, when supplemented into uncontaminated effluents or dilution water, the amount of offspring per adult daphnid decreased with increasing AgNP concentration. Interestingly, the highest test concentration of 100 μg/L showed no significant effect on the reproduction of the daphnids. These results can be explained by the transformation of AgNPs during their passage through the WWTP and potential agglomeration of AgNPs at high concentrations in the test media. Regarding the responses at the biochemical level, though significant changes were observed for daphnids exposed to the AgNPs-free effluent, in general, the presence of pristine and transformed AgNPs in the effluent did not significantly alter the measured parameters comparatively to the control effluent.

MO133

Multi-generational effects of silver nanoparticles in contaminated algae and from wastewater treatment plant effluent on reproduction and mortality in Daphnia magna.

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Silver nanoparticles (Ag-NPs) are increasingly becoming an environmental concern due to their widespread use in many products such as textiles, cosmetics, wound dressings and antimicrobial coatings. They reach aquatic ecosystems through waste-water treatment plants, accumulate and can still be toxic to aquatic organisms after being treated. Some studies have investigated long-term effects of manufactured nanomaterials, but have used only these materials in pure form, and few have studied the effect of Ag-NPs in contaminated algae. Our aim is to reflect a more realistic food chain, by studying how Ag-NPs in pure form (NM300K), Ag-NPs from effluent of wastewater treatment plants (WWTP) and Ag-NP contaminated algae affect reproduction and mortality of Daphnia magna over multiple generations. We selected Daphnia magna as it is a key species in a typical freshwater system, and a well-established model species for ecotoxicological studies. In our study, we performed three different exposure scenarios: (a) pure Ag-NPs (NM300K), (b) Ag-NPs from WWTP, and (c) algae contaminated with Ag-NP. In all three experiments we exposed the Daphnia to four concentrations of Ag-NPs (1.25μg/L, 2.5μg/L, 5.0μg/L and 10.0μg/L) and to solvent control (NM300K DIS) according to OECD guideline No. 211. The experiments lasted over five generations, with each generation lasting 21 days. We started each new generation with the third brood from the previous generation. For the statistical analysis we measured the survival and reproductive success to identify any differences between the exposure scenarios. We additionally measured body size at the end of each generation. Our results will give an overview of the toxicity potential of Ag-NPs from WWTP and those in contaminated algae in comparison to pure Ag-NPs, and so allow a more realistic risk assessment of Ag-NPs for the aquatic environment.

MO134

Effects of silver nanoparticles on the freshwater snail Physa acuta

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Silver nanoparticles (AgNPs) have been widely used in several commercial products, most likely ending up in aquatic ecosystems. Since the release of AgNPs into the aquatic ecosystems is a real scenario, it is mandatory to understand their potential impacts on aquatic organisms. The hermaphroditic freshwater snail *Physa acuta* was chosen as a non-target aquatic organism to evaluate the potential toxic effects of AgNPs (as pristine and as Ag_2S) due to their vital role in the aquatic ecosystem as efficient grazers and detritivores. Acute tests (96h) with juveniles (< 1mm) and adult (≥ 5 mm) snails were carried out to assess lethality. Chronic test (14 days) with egg masses were also performed to assess the effects of AgNPs on hatching success and embryo mortality. Acute AgNP toxicity increased with decreasing shell length; therefore, juvenile snails were more sensitive than adult snails. In the chronic test, the hatching decreased as AgNP concentrations increased. Embryo mortality was elevated at the highest tested AgNP concentrations. After the hatching, no mortality was observed. Some malformations were induced by AgNP exposure such as edemas and malformed shells. Ag_2S NP presented generally lower toxicity than pristine ones. Our study showed that AgNPs can indeed impair the life cycle of the freshwater snail *Physa acuta*, especially in the early stages of development such as hatching. **Keywords:** nanomaterial, *Physa acuta*, egg hatching, life cycle

MO135

Maternal Effects of Silver Nanocolloids on Fish Reproduction using Medaka

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To improve quality of human life, large amount of silver nanomaterials including silver nanocolloids (SNCs) were used for consumer and medical care products. The massive production and use of silver nanomaterials pose risk of contamination of aquatic ecosystems. Fish is the top predator in aquatic ecosystem. Chemical contamination of aquatic ecosystem and damage on fish provide us how environmental health status is, and also raise us how fish deals with uncomfortable environment. Early life stage of fish (egg and larva stages) is well known to be the most susceptible to xenobiotics, and then damage of xenobiotics in this stage should influence on later growth and reproduction; meanwhile, it is recognized that maternal effects of xenobiotics on offspring are also important for population growth. In this study, we exposed SNCs to medaka parents (2 male and 1 female) and observed effects on fecundity, sex ratio of offspring, and then assessed population growth. Concentration of SNCs exposure was gradually increased to see dose-dependent effects from 0.05, 0.5 and 5.0 mg/L. Before SNCs exposure, 10 groups of medaka parents were acclimatized in each 1 L of embryo rearing medium (ERM) under 16:8-h light:dark cycle at 25 ± 0.5 ºC for 13 days. Spawned eggs were harvested every day, and then fertilized eggs were counted. Fertilized eggs were incubated in ERM until hatch under the same condition above, and days to hatch and number of hatched larvae were also counted. After acclimation, firstly, 5 groups of medaka were subjected to exposure to 0.05 mg/L of SNCs in ERM (1 L) for 11 days, and the rest of 5 groups were incubated in ERM as control. Secondly, concentration of SNCs was increased to 0.5 mg/L for 11 days, and then finally increased to 5.0 mg/L for 11 days. During SNCs exposure periods, fecundity of exposed medaka was estimated. In exposure of 0.05 mg/L, 0.5 mg/L and 5.0 mg/L of SNCs, numbers of spawned eggs and fertilization ratio were declined; *i.e.* 26.8 ± 2.4 (eggs/female/day) and 82.1 ± 2.1 (%) in 0.05 mg/L, 22.1 ± 2.9 (eggs/female/day) and 34.2 ± 5.8 (%) in 0.5 mg/L, and 24.9 ± 2.9 (eggs/female/day) and 43.9 ± 3.7 (%) in 5.0 mg/L, which were lower than 32.3 ± 0.9 (eggs/female/day) and 87.8 ± 3.2 (%) in control. In the presentation, we will demonstrate maternal effects of SNCs exposure on reproduction with data of gonadal accumulation of SNCs, histological diagnosis, and other fecundity.

MO136

Titanium dioxide nanoparticles induces changes in antioxidant system and histopathology on gills and kidneys of fish, Prochilodus lineatus

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The present study investigated the toxicity of titanium dioxide nanoparticles (NP-TiO_2) in gills and kidneys of the Neotropical freshwater fish, *Prochilodus lineatus*, with emphasis on antioxidant responses and morphological damage. *P. lineatus* were exposed to 0 (control), 0.5, 1.5, 3.0 and 15 mg L^{-1} NP-TiO_2 for 48 h (acute exposure) and 14 d (subchronic exposure). In the gills, the reactive oxygen species (ROS) production decreasing and the glutathione (GSH) levels increasing showed concentration-dependent response, the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-Transferase (GST) as well as lipid peroxidation level did not change, after acute exposure. After subchronic exposure, ROS increased and SOD activity decreased after exposure to 15 mg L^{-1} NP-TiO_2; GSH levels increased after exposure to NP-TiO_2 concentrations higher than 1.5 mg L^{-1}. Hypertrophy, hyperplasia of filament and lamellar epithelium, epithelial lifting, dilatation of pillar cells system and blood congestion were the main histopathological changes mainly after subchronic exposure. Mitochondria-rich cells exhibited hypertrophy and hyperplasia were also observed only after subchronic exposure. The histopathological indexes indicated normal gill structure after acute exposure and increased after subchronic exposure indicating slightly to moderate damage. In the kidney, acute exposure to NP-TiO_2 did not result in ROS formation or changes in antioxidant enzymes, except for GSH and LPO which levels increased. After subchronic exposure, the CAT activity decreased. Histopathological changes were characterized by cellular hypertrophy, macrophages, nuclear and tubular degeneration after acute exposure and mainly by cellular and nuclear hypertrophy and melanomacrophages after subchronic exposure. The histopathological indexes indicated slightly to moderate tissue damage. GSH might play an important role in oxidative stress prevention on gills and kidneys of *P. lineatus*. Furthermore, the changes in gill structure after subchronic exposure may affect the gas exchange in this species.

MO137

Interaction of oxidized industrial multiwalled carbon nanotubes and cadmium using zebrafish cell line: influence of co-exposure protocols in toxicology studies in vitro

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There is a lack of understanding about the behavior and potential risks of the increasing presence of nanomaterials in the environment. Furthermore, the potential risks of nanoparticles associated with heavy metals have not been well known and standardized, particularly in fish cells. In this context, this study evaluated the interaction of oxidized multiwalled carbon nanotubes (MWCNT) with cadmium using zebrafish cell line (ZFL). The focus was to evaluate the sample preparation towards co-exposure to *in vitro* models. Autoclaved ox-MWCNT thermal stability was evaluated by thermogravimetric analysis. Morphology was observed by scanning electronic microscopy and transmission electronic microscopy. Chemical surface analysis was carried out on a K-Alpha photoelectron spectrometer (XPS) system. Average hydrodynamic sizes and zeta potentials were evaluated by dynamic light scattering and electrophoretic light scattering. Two protocols for MWCNT stabilization were performed and monitored by measuring the optical density at 400 nm for 0, 1 and 24h and three treatments are applied on cells: (1) a dose-response curve was performed with Cd^{2+}, (2) MWCNT and medium RPMI containing 10% of fetal bovine serum (FBS) for 30 minutes and thereafter Cd^{2+}, and (3) MWCNT and Cd^{2+} for 30 minutes and then RPMI and 10% FSB medium. Cytotoxicity assays were performed using Trypan Blue, Neutral Red, LDH leakage, MTT salt and apoptosis and necrosis rates. Morphological analysis and cellular confluence were also performed. This study evidenced that the order of compounds on the interaction MWCNT and Cd^{2+} interfere on the toxicological effects of *in vitro* studies. Despite the MWCNT did not cause toxicity on ZF-L cells in some toxicity tests, the material induces apoptosis and necrosis and intervene on cadmium toxicity. The presence of FBS on protocols alter cadmium toxicity and changes the colloidal behavior of MWCNTs. The complex formed after interaction MWCNT-metal present a false viability result by neutral red probably due the increased formation of endosomes and/or enlargement of lysosomes. Further researches on standardization methods and reproducible and effective protocols for evaluating the cytotoxicity of carbon nanotubes, in particular, during co-exposure with environmental pollutants is needed.

MO138

Effects of silver nanoparticles on Folsomia candida (Collembola) reproduction in different standard soils and in long-term aged sludge-treated soils

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Soils are considered a major sink of silver nanoparticles (AgNP) because AgNP enter these by application of sewage sludge from waste water treatment plants to agricultural soils. Due to the rising use of AgNP, long-term emission to soils and long-term accumulation are expected. In this study, the effect of AgNP (NM-300K) and $AgNO_3$, as a metal salt reference, in three standard soils (OECD, RefeSol 01-A, Lufa 2.2) on *F. candida* reproduction at low (μg Ag kg^{-1} range) and medium (mg Ag kg^{-1} range) concentrations was determined. In addition, to simulate realistic exposure pathways, effects on *F. candida* reproduction after AgNP application to soil via sewage sludge and after aging this treatment in the soil for up to 140 days were studied using environmentally relevant concentrations of AgNP. The generated data demonstrate that the presence of AgNP in the soil in the low mg Ag kg^{-1} concentration range results in significant, but concentration independent inhibition of *F. candida* reproduction. Toxic effects were found in RefeSol and Lufa soil but not in OECD soil. The toxicity did not differ between RefeSol and Lufa in this concentration range. Significant inhibition of *F. candida* reproduction due to NM-300K and $AgNO_3$ was also observed for soil amended with AgNP treated sludge. An increase in inhibition with aging of the AgNP and $AgNO_3$ in the soil was evident. This effect was more pronounced and more consistent for AgNP than for $AgNO_3$ and was detected only after aging of 60 days and longer. In conclusion, long-term experiments reveal an increase in the toxicity of NM-300K on *F. candida* with aging at environmentally relevant concentrations and realistic exposure scenarios. Consequently, the consideration of transformations and the implementation of long-term tests when performing the environmental risk assessment of AgNP (and other nanoparticles) are essential.

MO139

In silico approaches for the prediction of the cytotoxic activity of heterogeneous nanoparticles

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The COST Action TD1204 'Modelling Nanomaterial Toxicity' (MODENA) project was established in 2012 to promote cooperation between researchers and regulators on the topic of computational nanotoxicology (http://www.modena-cost.eu). A main output of MODENA was the creation of a large dataset with citotoxicity data measured for 192 heterogeneous nanoparticles using different assays (i.e ATP, LDH and MTT+WST-1) in various cell lines, intended for *in silico* analysis. Quantitative descriptors, either derived from the periodic table or describing experimental conditions and structural properties (e.g. time of exposure, cell typology, serum concentration, primary size, aspect ratio..), were included as input variables to generate Quantitative Structure-Activity Relationships (QSAR) models. In this poster we summarize results obtained by different partners of the MODENA project by applying different modelling approaches to the same dataset. Multiple Linear Regression models based on the Ordinary Least Squares method (MLR-OLS) were developed in the software QSARINS, i.e. Genetic Algorithm (GA) optimized for MLR was applied for the selection of most relevant modeling variables. The models had good fitting ability (R^2 mostly > 90%) and internal robustness (Q^2_{loo} and $Q^2_{lmo30\%}$ ranges 75% and 90%). Additionally, we trained two different nonlinear regressors (Support Vector Regressors (SVR) with polynomial kernels and Radial Basis Function (RBF)) within a nested-cross validation scheme for parameter optimization while using "best first" strategies as well as a greedy stepwise search method for feature selection. Most regression learning systems achieved R^2 values above 90% and the correlation between real and estimated toxicity endpoint values increased monotonically with the number of included features. With both linear and nonlinear approaches, structural features as well as experimental conditions variables e.g. time of exposure, primary size and aspect ratio were selected as the most relevant varialbes to model NPs activity. These trends did not change significantly between toxicity endpoints. In this study we confirmed the scientific relevance of the dataset created within the MODENA COST Action from data generated independently by different laboratories. The consistency of the information included in the data set was the basis for the quantification, through matematical models, of robust structure-activity relationships.

MO140

Supporting the development of safer nano-based formulations for the restoration of modern and contemporary works of art

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Currently there is a lack of methodologies for the conservation of modern/contemporary artworks, many of which will not be accessible in short time due to extremely fast degradation processes. In this context the application of nanotechnology already showed promising results and more solutions are currently being explored in the frame of the NANORESTART (NANOmaterials for the REStoration of works of ART) H2020 project. In particular the project aims at developing nano-based formulations to ensure long term protection and security of modern/contemporary cultural heritage, taking into account environmental and human risks, feasibility and materials costs. Specifically, it focuses on: (i) tools for controlled cleaning, such as highly retentive gels for the confinement of enzymes and nanostructured fluids based on green surfactants; (ii) the strengthening and protection of surfaces by using nanocontainers, nanoparticles and supramolecular systems/assemblies; (iii) nanostructured substrates and sensors for enhanced molecules detection. To support the developers of new materials in designing safer products, a three steps strategy was designed and is currently adopted in the project. First, a list of potential ingredients (along with their Material Safety Data Sheets) is collected for each proposed innovative formulation together with its composition (as ranges of percentage in weight for each ingredient). This allows to apply the self-classification approach included in the EU Classification, Labelling and Packaging (CLP) regulation with the aim to classify each formulation according to a set of human health and environmental hazards and to advise the material developers about weight thresholds that should not be exceeded in order to avoid specific hazards. Secondly, information on conventional counterparts already in the market (including their Safety Data Sheets) are collected in order to prioritize the most promising formulations to work with and in particular those on which physico-chemical characterization in environmental and biological media as well as (eco)toxicological testing will be performed. Estimated toxicity will then be compared to the results of the self-classification approach in order to highlight cases in which nano-specific hazards can be pointed out. Results are supporting the third and last step in which environmental impacts along the life-cycle of the formulations (in particular in application and post-applications stages) are assessed.

MO141

SETAC Nanotechnology Interest Group

C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology

Persistence & Biodegradation Assessment (P)

MO142

Soil persistence of the anionic surfactant sodium lauryl ether sulphate in two different foaming agents used for a mechanized tunnelling

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The anionic surfactant sodium lauryl ether sulphate (SLES) is the main component of most commercial products used for soil conditioning in the excavation industry, in particular as lubricants for mechanized tunnelling. Its use during the excavation processes can result in either the subsequent possible re-use of the huge amount of soil debris as by products (e.g. land covering) or its discharge as waste. Currently, there are neither SLES soil threshold limits in European legislation, nor comprehensive studies on the environmental risk for soil ecosystems in these exposure scenarios. Although SLES is generally reported to be biodegradable in standard tests, data on its biodegradation in environmental studies are quite scarce. Consequently, assessing SLES biodegradation rates in field conditions is crucial for evaluating if in residual concentrations it can or not be a potential hazard for terrestrial and water organisms. In this context, a set of microcosms was set up using soil samples from a construction site. Soil samples were treated separately with one of two commercial products (P1 and P2) in absence/presence of lime at the concentrations used for the mechanized drill. Lime treatment is used because chemically transforms unstable soils into usable materials, making it possible their appropriate use for land covering. At selected times (0, 7, 14, 21, 28 d) soil samples were collected for assessing SLES concentration in the different conditions. Moreover, microbiological analysis were performed in order to assess microbial abundance, cell viability, dehydrogenase activity and microbial structure. The SLES degradation rates were higher when SLES was present in the P1 (DT_{50}=11 d) foaming agent than in the P2 (DT_{50}= 17d) one. Lime addition slowed down SLES halving in P1 (DT_{50}= 31 d), while inhibited its degradation in P2. In line with these results, the bacterial community was differently affected by the foaming agent P1 or P2 both in presence and absence of the lime. The overall results show SLES as a biodegradable compound and that the different formulation of each specific foaming agents (P1 or P2) can affect its persistence.

MO143

Assessing chemical persistence in marine water using biodegradation screening and simulation tests: impact of the test conditions on the results and the regulatory decisions

G. Deviller, SOLVAY / TERA PRAPS HSE; P. Chagnon, SOLVAY / Research and Innovation

In order to assess the persistency of substances in marine waters, biodegradation screening and simulation tests according to the standard OECD guidelines are commonly required by the authorities. However, recent results from CEFIC-LRi ECO11 and ECO18 funded projects have shown that the test conditions could have a major impact on the estimated biodegradation rates and, as a consequence, on the regulatory decisions for the tested substances. In 2016, an OECD 306 ring test exercise had been launched in order to determine if the current methodology can be improved and, a new temperature of 12°C is now recommended under REACH regulation for simulation test. In order to assess at which extent the test design is affecting the biodegradation results in marine water a series of tests according to the OECD 306 test guideline have been launched with different exposure time, temperature, water replacement regime, water volume, parent/metabolite substance and nitrogen source. Further, an OECD 309 simulation test with surface water has been performed in order to confirm the previous results and complete the biodegradation pathways with radio-labelled substances. The results of the screening and simulation tests are statistically assess to determine the key parameters influencing biodegradation in marine water. They will also be critically compared according to their environmental relevance and their regulatory consequences under various chemical regulations.

MO144

Persistence Assessment of Fragrance Ingredients in the Aquatic Environment through Photodegradation Studies

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Photodegradation is an important abiotic degradation process to be taken into account for the regulatory evaluation of environmental risk and hazards of chemical substances, especially poorly biodegradable ones. Photodegradation may occur via direct and indirect mechanisms. In direct photodegradation, the absorption of a photon by a chemical results in bond cleavage or rearrangement to form new chemical species. In indirect photolysis, a chemical is degraded by a series of reactive species generated from photolysis of other substances present in the water, such as dissolved organic matter (DOM). Based on standard guidelines for photodegradation, we developed workflows to study photodegradation of fragrance ingredients for their persistence assessment in the aquatic environment. Case studies for direct photodegradation of Myrrhone® Neo and indirect photodegradation of Pamplewood will be presented. Direct photodegradation quantum yield of Myrrhone® Neo was determined to be 0.097 ± 0.009 in the laboratory using PNA/pyridine as an actinometer. Direct photodegradation half-lives of Myrrhone® Neo in natural waters were estimated as a function of season and latitude based on the quantum yield. Three main volatile photodegradation products, which continued to photodegrade, were observed. They were tentatively identified based on their EI mass spectra and photochemical reaction mechanism. In addition, their environmental profiles were assessed by modeling. Indirect photodegradation rates of Pamplewood under various indoor and outdoor conditions were measured using an LC-MS method. Although the half-life varied from 4 to 13 days, it collectively indicated that Pamplewood is intrinsically photolabile and can undergo rapid photodegradation. Results from quencher experiments revealed that ×OH was the main reactive intermediate responsible for indirect photodegradation, resulting in a Pamplewood half-life of about 18 days in sunlit surface water, based on the experimentally determined second-order rate constant ($8.48 \pm 0.19 \cdot 10^9 M^{-1}s^{-1}$). Photodegradation products were studied by GC-MS whereby intermediates of Pamplewood continued to photodegrade. Complete mineralization was observed by total organic carbon analysis when Pamplewood was reacted with hydroxyl radicals in an aqueous solution. It is concluded that abiotic processes should be considered in the persistence assessment of chemicals in the aquatic environment.

MO145

Combined MALDI-TOF MS Imaging and LC-HRMS study of biodegradation in solid polycaprolactonediol exposed to different wastewater environments

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MALDI IMAGING MS (MSI) consists on the use of matrix-assisted laser desorption ionization as a mass spectrometry imaging technique. The sample is scanned in two dimensions at a preselected spatial resolution while the mass spectrum is recorded. Advantages, like measuring the spatial distribution of a large amount of analytes at one time, make it a useful method in solid-surface studies. Waste Water Treatment Plants (WWTP) are constituted by a sequence of various process steps designed to remove both organic waste and nutrients. Typically, they include a secondary aerobic (biological) treatment aimed at eliminating organic load, followed by a tertiary treatment (nitrification/denitrification) process to remove nitrogen. Each treatment step involves different specific conditions (aerobic or anoxic), which result on the development of adapted microorganisms consortia. In the present contribution, we studied the behavior of a synthetic polymer exposed to these different aquatic WWTP environments. Polycaprolactone (average MW=1250) was selected as suitable candidate polymer probe. The method was tested in the WWTP of Barcelona, El Prat de Llobregat by exposing the polymer probes *in situ* at different environments, namely, the secondary and nitrifying/ denitrifying conditions. Progress of biodegradation after several days of exposure was reflected on changes in the MALDI-TOF MSI mass spectra obtained across the sample's surface. Peaks were identified using liquid chromatography coupled to high resolution mass spectrometry (QExactive, Thermo Scientific). Results were further processed using different image processing tools. In order to gain some insight on the microorganisms responsible of polymer degradation, of bacterial communities attached to the polymers and those free-living in the different WWTP treatments were carried out by means of 16S amplification and MiSeq Illumina sequencing. **References** 1. Rivas D, Ginebreda A, Pérez S, Quero C, Barceló D. MALDI-TOF MS Imaging evidences spatial differences in the degradation of solid polycaprolactone diol in water under aerobic and denitrifying conditions. Science of The Total Environment 2016b; 566–567: 27-33.

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MO146

Regulatory assessment of environmental persistence: OECD biodegradability screening tests – need for improvements and possibilities for enhancements

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A literature study was performed to review the applicability of established and new tests on biodegradability for assessing persistence in the frame of PBT/vPvB assessments under REACH. The focus was on existing biodegradability screening tests, which were systematically analysed for strengths and weaknesses. Due to their history, tests on Ready Biodegradability show differences with regard to (beyond others) test conditions and validity criteria which cannot be explained scientifically but which impact probability of biodegradation for a given compound. Proposals are made how to improve their performance and suitability for assessments of persistence considering relevant scientific literature. Some recommendations are also given for simulation tests (OECD 307, 308, 309). Screening tests on ready biodegradability are purposely conservative, such that in case of failing criteria for ready biodegradability substances may require further investigations for PBT/vPvB assessment under REACH. "Enhancements" for "Ready-Tests" as well as newly developed tests aim at reliable conclusions on persistence without the difficulties associated with performance of simulation tests. It is critically discussed which test modifications could be introduced without challenging the screening nature of these tests. Furthermore, specific issues such as substances difficult to test in standard tests (poorly water soluble, highly volatile or UVCB sub-stances) are addressed. Within a 2-(half)-days' workshop and expert discussion at the venue of the German Umweltbundesamt (UBA) with participants from different insistutions (e.g. EU Member States Competent Authorities, EC DG Environment, ECHA) project results were discussed. Important agreed conclusions are summarized. Gartiser, S.; Schneider, K.; Schwarz, M.A.; Junker, T. (2017)\n Assessment of environmental persistence: regulatory requirements and practical possibilities – available test systems, identification of technical constraints and indication of possible solutions.\n Final Report, including documentation of the workshop. UBA-Texte 10/2017\n https://www.umweltbundesamt.de/publikationen/assessment-of-environmental-persistence-regulatory \n

MO147

Ring test to improve the OECD 306 marine biodegradation screening test

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A series of international standardised tests have been approved by the OECD to measure the relative biodegradability of substances. Among these tests, biodegradation screening tests (BSTs) form the first tiers of assessment, offering relatively simple and cheap characterisations of biodegradability. Most parameters in these BSTs are highly prescribed and conservative, but the microbial inoculum is the least controlled parameter. The resulting high levels of variation and false negative results have been recognised as a limitation since the introduction of these tests up to today and are especially reported for the marine BST OECD 306. BSTs were designed over two decades ago and do not, in their current form, fulfil the present requirements anymore. In recent years, regulatory emphasis has shifted from identifying chemicals that are rapidly biodegradable to identifying chemicals that are potentially persistent in the environment. Technical guidance documents, which have been prepared under the European chemicals regulation system known as REACH, have suggested several improvements to effectively assess persistence with BSTs. Within their nature, these modifications and enhancements also address a number of the commonly discussed reasons for high variability and poor reliability of BSTs. The Cefic LRi ECO11 project investigated and validated several enhancements for the marine BST. It was possible to represent the bacterial diversity in the sampled environment better by increasing the microbial inoculum to environmentally relevant concentrations through tangential flow filtration. This standardisation led to a significant decrease in variability between replicates in comparison to the standard marine BST. By extending the test duration beyond the persistence half-life threshold, previously reported elongated biodegradation lag phases for chemicals in surface water were recognised. A ring test project started in 2016 to validate these intra-laboratory findings from Cefic LRi ECO11 in other testing facilities within Europe, North America and Japan. Academia, industry, CROs and regulatory bodies are cooperating to improve the marine BST OECD 306. A summary of the test protocol together with preliminary test results and the status of the experimental work will be presented.

MO148

Using EFSA Regulatory Data to Explore Pesticide Biodegradation Half-life Variability

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Microbial biotransformation is the primary route of degradation for most pesticides in soils. EAWAG-SOIL is an unprecedentedly rich database package of enviPath which contains data on aerobic degradation in soil for 275 pesticides which we manually extracted from European Food Safety Authority (EFSA) dossiers, curated, and made publicly available (Latino et al., 2017; envipath.org). Its defining features, besides currently containing 4716 non-redundant experimental half-life (DT50) values for parent pesticides and metabolites are: (i) the availability of corresponding metadata on experimental conditions such as pH, temperature, and soil properties, and (ii) electronically encoded information on the observed biotransformation reactions. Analysis of the DT50 values showed that individual pesticides have DT50 ranges of up to 3 orders of magnitude, just one order lower than the entire range of median DT50 values spanned by all pesticides. Therefore, it will be essential to consider the influence of experimental conditions on degradation half-lives in order to build accurate and robust prediction models of biotransformation. To understand the potential dependencies between DT50 and experimental/environmental conditions, bivariate analyses were performed within groups of pesticides classified according to chemical structure and initial biotransformation reaction, the underlying hypothesis being that compounds with common functional groups and/or similar initial biotransformation reactions would show similar dependencies between DT50 and experimental conditions. Complementary to the bivariate explorative analysis, we also explicitly corrected observed DT50 values for differences in bioavailability according to Honti et al. (2016) to test the hypothesis that part of the observed intra- and intercompound variability in DT50 values is due to differences in bioavailability. In this way, we (i) interrogate the effect(s) of individual factors on DT50 through bivariate analysis, and (ii) develop an understanding of which chemical properties, be they structure, initial reactivity, and/or physicochemical properties, exert a dominant influence on DT50. These aspects form a solid basis for multivariate regression and constructing other more complex prediction models, which form the scope of our future work.\nLatino et al. (2017), Environ. Sci.: Processes Impacts, 193(3), 449-464.\nHonti et al. (2016), Environ. Sci. Technol., 50(13), 6865-6872.

MO149

The effect of aging seawater on microbial communities before preforming biodegradation tests according to OECD test guideline 306

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Biodegradation of organic chemicals in seawater, using the closed bottle method, is dependent on a diverse microbial community to be present in the natural seawater that is used as the test medium. However, there has been some concern about the reproducibility of this test partly because of variable results obtained by different laboratories. The OECD 306 test guideline indicates several methods for pre-treatment to be used, including aging. The hypothesis is that aging the seawater before use, especially in the cold season, will aid acclimating of the microbial community, selecting for the microorganisms that can survive under laboratory conditions. If acclimation of the seawater is performed in storage vessels, the microbial diversity should be higher and more uniform than if the acclimation is carried out in small test bottles, and then the lag-phase of the test will be shorter. This study was preformed to document the effect of sampling depth, aging (1 to 7 days, with or without nutrients) and incubation temperature (20 or 15?C) on the abundance, diversity and degradation rate of the microbial communities in a closed bottle test using the reference substance aniline. The seawater was sampled in November from the outer Oslo fjord when the temperature is much lower than the test temperature, and both surface water and deep water (60m) (which is cleaner and might have a more stable microbial community) was used. Degradation of aniline was assessed by measuring dissolved oxygen, and the concentration and “community fingerprint” of bacteria was measured by flow cytometry. The biodegradation of aniline improved after aging the seawater; the lag-phase was shorter and the reproducibility of the test was improved. Deep water produced better and more stable results than surface water. Incubation at 15?C resulted in much slower bioactivity and no degradation after 7 day’s incubation was observed (except for one bottle), while 20?C incubation gave up to 75% biodegradation. The concentration of bacteria in the test water increased during the first 4 days of storage, and stabilized after 6 days. The concentration was higher in the storage tank than in the blank bottles after 5 to 8 days of storage, indicating that the larger volume in the storage tank (5-20 litres) compared to the bottles (0.3 litres) encouraged microbial diversity. The diversity of the bacteria also changed during storage, and both diversity and concentration changed when biodegradation took place.

MO150

Assessing chemical persistence for "difficult substances" using latest developments in biodegradation screening and simulation tests

G. Deviller, SOLVAY / TERA PRAPS HSE; R. Patoux, SOLVAY; M. Enrici, SOLVAY / HSE - PRA PS

In order to assess the persistence of substances, biodegradation screening and simulation tests conducted according to the standard OECD guidelines are commonly required. However, these methods are not always applicable for “difficult substances” (toxic to micro-organisms, low soluble, multi-constituent…) and recent results from research initiatives (e.g. CEFIC-LRi ECO11, ECO18, ECO24 and ECO 25 projects) have shown that the test conditions can have a major impact on the estimated biodegradation rates and the prediction of the degradation pathways. Therefore, adapting the test design to the characteristics of the substances is a key element that may drive the study outcome and consequently the regulatory assessment. The presentation will show several tests results coming from adaptations of biodegradation tests designs to address persistence assessment of difficult substances. More specifically, the impact of test substance concentration and degradation state on its toxicity to the inoculum was assessed in OECD screening tests (e.g. OECD 301, OECD306). Other results show how an enhanced CO2 evolution biodegradation test with longer duration and the use of Bioavailability Improvement methods (BIM) can be applied to insoluble substances and UVCB. Finally, results from biodegradation simulation tests in STP (OECD 314), surface water (OECD 309) and soils (OECD 307) will be presented in order to define how they can support the persistence assessment of toxic and/or low soluble substances ensuring environmental relevance. Consistency with screening test results and formation of Non Extractable Residues will be covered as well as the relevance of the new temperature of 12°C recommended by the REACH regulation for PBT assessment. All these elements related to “difficult substances” can serve the strategies for PBT assessment and the revision of the PBT guidance document.

MO151

OECD 306: Investigation of alternative test designs for testing the biodegradability in seawater

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Within persistency assessment biodegradation in seawater is a topic of increasing interest. The established TG OECD 306 offers two alternative test designs for investigating the biodegradability in seawater, each with its own advantages and limitations. The shake flask method has the inherent disadvantage of only allowing to determine the elimination of a substance by measuring the DOC. This renders this method unsuitable for adsorbing, insoluble or volatile substances. The alternative closed bottle method allows to determine the biodegradation by measuring oxygen consumption. Nevertheless, the limited amount of dissolved oxygen within the bottle makes it prone to missing the validity criteria in case of higher background activity, especially when nitrifying bacteria are present. Therefore, substances with certain characteristics can only be subjected to the test acc. to OECD 306 with restricted bioavailability or not at all. This might raise high costs when substances for that reason have to be tested in expensive simulations tests. Looking for alternatives to overcome some of the shortcomings of the TG OECD 306, the TG OECD 301 for ready biodegradation offers a number of test designs, of which the OECD 301 A/D and E, respectively, in principle resemble the methods from TG OECD 306. The method OECD 301F (Manometric Respirometry) allows to measure biodegradation by determination of the oxygen consumption. Due to the headspace, oxygen is not as limited as in the closed bottle method. This makes this setup less sensitive for higher background activities. Since the test procedure is less circumstantial in the respirometry method, this gives more room for pre-treatment and bioavailability improvement for difficult substances. The continuous measurements allow for a more precise description of the biodegradation curve without increasing the number of replicates. Data on biodegradation of the reference item sodium benzoate in the manometric respirometry method with seawater will be shown in comparison to the original TG OECD 306 methods. Also the suitability of the OECD 301B Modified Sturm Test design will be briefly discussed.

MO152

How important is sediment microbial diversity for degradation of pharmaceuticals?

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Pharmaceuticals are ubiquitous in aquatic systems affected by waste water treatment plants (WWTPs), where they can undergo further degradation. This process is determined not only by the compound's physicochemical properties, but also environmental factors. For instance, the attenuation rate of acetaminophen differed by a factor of 40 between rivers Gründlach (Germany) and Fyris (Sweden) [1]. Although data is limited, previous studies indicate that biodiversity may be linked to the potential for degradation of micropollutans [2]. However, the recommended biodegradation tests (i.e. OECD TG308) include no information on the microbial community. We designed an experiment based on OECD TG308 to assess the influence of bacterial diversity on pharmaceutical degradation. Water and sediment were collected from rivers Fyris and Gründlach, before and after the discharge of the local WWTP. These rivers have divergent hydraulic boundary conditions, wastewater proportions and hence anticipated differences in biodiversity. The four sediments were acclimated with synthetic river water for 10 days, then spiked with a mixture of 11 pharmaceuticals at two concentration levels (200 μg/L and 2 mg/L) to test the response of the microbial community in the sediment. The selected compounds represent a wide range of biodegradation rates: acetaminophen, acesulfame K, caffeine, carbamazepine, diclofenac, furosemide, ibuprofen, metformin, oxazepam, tramadol and venlafaxine. During the 40-day incubation, the bottles were kept in the dark at 16°C and aerated daily to maintain an aerobic water column with an oxygen gradient in the sediment. The water phase was sampled at 10 time points while sediment samples were taken after acclimation and at the end of the test. We will measure the pharmaceutical concentrations in all samples, and use Illumina sequencing of bacterial 16S rRNA to analyze the active bacterial community composition. Rényi's entropy profiles will be calculated to test whether differences in richness, eveness and community composition can be associated with the degradation potential. Our results will show whether microbial communities up- and downstream of a WWTP differ in their potential to degrade pharmaceuticals and whether differences in the composition and diversity can explain the variation in the degradation rates of micropollutants between sample sites and rivers. [1] Li et al. 2016. doi: 10.1021/acs.est.5b06327 [2] Johnson et al., 2015. doi:10.1128/AEM.03286-14

MO153

The impacts of light and flowing water on isopyrazam dissipation in river microcosms

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Industry use microcosm scale regulatory tests as a rapid and cost effective indication of how a chemical transforms in the environment. There is, however, uncertainty over how the data from these tests can be extrapolated to the environment. Larger more realistic studies could help bridge the gap between the behavior of chemicals in these small-scale tests and the environment, and indicate how small-scale tests could be modified to improve their predictive power. Here, we investigated the impact of non-UV light and water movement (static and flowing) on the dissipation of a fungicide, isopyrazam, in controlled environment glass flume systems (40 L total capacity). Water and sediment (sieved to < 2 cm) collected from the River Dene (Wellesbourne, UK) was transferred to flume systems (12 cm water depth and 3 cm sediment depth). Isopyrazam was added to the flumes at a concentration of 0.1 mg/L, and recovery in water and sediment determined over a 52-day sampling period using LC-MS. Additional to this, microbial and water chemistry analysis was also carried out. Significant dissipation of isozpyrazam occurred in all systems, however it was quicker in flowing treatments than static. No significant difference was observed in light-dark cycles. This was potentially due to an increase in mixing in these systems, causing microbial degraders to come into contact with the compound more frequently. In the static systems, dissipation occurred quicker in the light than in the dark, suggesting that phototrophs, in addition to heterotrophs, were involved in dissipation. Previous water-sediment studies of isopyrazam degradation in small-scale microcosms did not show degradation under dark conditions, and light was a major determinant of degradation. The flume system results suggest that different factors control the dissipation rate as scale and environmental realism of test systems change.

MO154

Modification of the Standard Laboratory Water Sediment Study using Sunlight to Refine the Estimated Environmental Concentration

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An agrochemical was observed to degrade rapidly in buffer and natural water in a photolysis screen while degradation in water sediment proceeded slowly. This suggested that photodegradation in the water phase may contribute to reduced persistence of the parent compound in the water sediment system. An aerobic water sediment study was conducted with the test compound in accordance with regulatory guidelines with incubation in the dark. An irradiated study was conducted similarly but incubated outdoors to permit exposure to natural sunlight where the formation and decline of transformation products and the persistence of the test compound in the total system could be evaluated. The test compound degraded more rapidly in the irradiated Sand and Silt systems than in the corresponding dark systems. A known photoproduct was observed in both the water and sediment phases of the Sand and Silt systems exposed to natural sunlight. The study in natural sunlight can serve as a good indicator that aquatic field (shallow pond or aquatic field dissipation) studies may provided additional refinement of environmental concentrations. For agrochemicals known to be photolabile, integrating natural sunlight into the guideline-compliant laboratory aerobic water sediment study can provide refinement to the estimated environmental concentrations

MO155

Application of chemostat systems for adaptation of microbial communities in persistency testing

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Persistency of chemicals is a key parameter for estimating the environmental risk of a newly synthesized chemical. Its degradation rate is determined in a range of laboratory-based microbial biodegradation processes, developed by the Organisation for Economic Co-operation and Development (OECD). Ready biodegradability testing (RBTs) has been designed as simple and non-expensive methods to identify chemicals that are not expected to be environmentally persistent. However, since the origin of the bacterial inoculum as well as the pretreatment are not standardized, the results of the current RBTs are not consistent. We hypothesize that this inconsistency is the result of different degrees of adaptation capacity of the microorganism in the biodegradation of chemicals. The aim of our project is to study microbial adaptation to persistent chemicals using long term exposure in various culturing systems in order to formulate guidelines for RBTs taking pre-adaptation of the microbes into account. This in turn might lead to a faster rate of degradation of chemicals that were initially persistent. Despite numerous publications demonstrating the impact of adaptation on biodegradation test results, microbial adaptation is not yet included in the guidelines. Our approach is to expose microbial communities from wastewater, growing in chemostat and batch culturing systems, to different persistent chemicals for a long term and under defined conditions. The biodegradation capacity of pre-exposed inoculum is assessed in RBTs and changes in community structure are followed by Illumina amplicon sequencing in time. Removal of tested chemicals and their transformation products are determined by LC MS/MS. Results of this experiment are expected to show biodegradation capacity enhancement of the inoculum after pre-exposure and diminishment of the variation in results of the RBTs. They will also result in a better understanding of the relationship between microbial adaptation and biodegradation performance and will ultimately facilitate the design of more robust screening studies and will allow a better prediction of the biodegradation outcome.

MO156

Investigations on microbiota composition of sludge inoculum in the presence and absence of carbon source in different OECD 301 series biodegradation screening tests

A. Boschung, Firmenich; S. Rey, Firmenich / Biotechnology; A. Siegl, C. Beimfohr, Vermicon AG; M. Seyfried, Firmenich

Sludge obtained from wastewater treatment plants (WWTPs) has been used as inoculum in OECD biodegradation screening tests for decades, serving as an indicator for breakdown of industrial chemicals in the environment. While numerous studies have demonstrated the complexity of sludge microbiota, little is known on how the composition can change throughout the course of incubation in different OECD screening tests and as a function of the presence of specific carbon sources. In most cases, screening tests are treated as a “black box” that do not allow any insights in underlying microbiological characteristics of the inoculum and test parameters that govern it. In order to reach a better understanding of how viability, composition and diversity of the inoculum microbiota behave under standard screening test conditions, and how specific groups of competent degraders might become predominant during screening tests, a culture-independent approach was chosen to monitor developments in OECD biodegradation screening tests. Monitoring was carried out by applying ribosome-specific probes targeting the viable part of the inoculum via fluorescent in situ hybridization (FISH). The approach of using up to 10 probes covering main eubacterial groups was previously shown to cover the prokaryotic diversity of unprocessed wastewater treatment sludge to a high extent (95-98%). In our hands, samples from inoculum preparations originating from a WWTP from a rural area of Geneva (La Vilette / Thônex) subjected to conditions typical of biodegradation testing in OECD tests showed significant differences in overall viability and cell numbers of main prokaryotic phylogenetic groups between OECD tests and throughout the 28-day incubation phase of individual tests, particularly after test compound was added. Excellent recovery of individual phylogenetic groups was confirmed compared to a non-specific, comprehensive eubacterial probe. Thanks to this monitoring approach, first insights in potential test-specific limitations were gained, and a better, more general understanding of the dynamics of microbiota over time in OECD 301 series screening tests was obtained.

MO157

Impact of the inoculum composition on ready biodegradability tests behavior

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Next generation sequencing provides better access to the structural and functional diversity of microorganisms. The bacteria of activated sludge from wastewater treatment plants allow the biotic degradation of chemicals released down the drain. In order to determine the ready biodegradability of chemicals, laboratory tests are performed with domestic sludge from treatment plants, which undergoes washing and dilution treatments before being used as an inoculum. The results obtained with these tests show a certain disparity, one of the major factors being the inoculum sample, which differs in each test. This variability of results increases when the test substance is poorly water-soluble, which makes it less bioavailable for bacterial biodegraders present in the inoculum. In this context, metagenomic analyses have been applied to follow the bacterial evolution of the inoculum from the wastewater treatment plant sample to the test and related to the inoculum’s capability to biodegrade. The first step aims to verify the effect of the pretreatment (sludge washing and dilution) and the selection pressure exerted by the test medium only on the inoculum composition. The second step concerns the impact of the bacterial composition of the inoculum on its capability to biodegrade the two poorly water-soluble substances.

MO158

Simultaneous biodegradation of water treatment additives: Transformation and crosslinked product formation, impact of biocide shockdosing and salinity

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Cooling towers account for a significant part of the industrial fresh water uptake. Several treatment technologies like reverse osmosis, electrodialysis and membrane distillation are employed to facilitate the reuse of discharged brackish cooling tower water. Next to a moderate salinity of ± 5 g/L, cooling towers water also contains different water treatment chemicals as corrosion inhibitors, biocides and antiscalants that are used simultaneously to maintain optimal functioning of the water circuit. However, these water treatment chemicals hamper the optimal functioning of the treatment technologies by for instance membrane fouling. An interesting water pre-treatment option is the use of natural treatment systems like constructed wetlands (CWs). Biodegradation is one of the main contaminant removal mechanisms in CWs. However, the biodegradation potential of CWs for many of the water treatment chemicals is not well understood. In this study, the simultaneous biodegradation of different representative water treatment chemicals by a CW inoculum is explored. The representative water treatment chemicals consist of 1H-benzotriazole (corrosion inhibitor), DBNPA (biocide), glutaraldehyde (biocide), PEG (surfactant) and HEDP (antiscalant). The following subquestions are addressed: - Does shock dosing with biocides affect the CW biodegradation potential for the target chemicals? - What is the influence of different salinities on the biodegradation of the target chemicals? - Which signature microbial transformation products are being produced by single target chemicals that can be used to monitor biodegradation in CW systems? - Do possible transformation products show ecotoxicological effects? - Does the simultaneous biodegradation of multiple water treatment chemicals result in the production of new possibly harmful crosslinked products?

MO159

Degradation of glyphosate in soil influenced by interaction between Collembola and soil microbial community

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Glyphosate is the most widely used herbicide because of its broad spectrum activity and effectiveness, however, little is known about adverse effects on non-target species and their interactions. Therefore, in this study, we investigated the effects of glyphosate on interactions between Collembola and soil microbial community and the effect of Collembola on degradation of glyphosate. The experiment carried out in PS container filled with 30g of soil according to OECD 232 guidelines. Investigating the effects of soil microbial community and Collembola on degradation of glyphosate, we prepared defaunated field soil (only maintaining soil microbial community, sampling in May and September, 2016.) and autoclaved soil with 0, 10, 30 adults of *Paronychiurus kimi* (Collembola) respectively. Survived adults and hatched juveniles of *P. kimi* were counted after 28-day exposures in both soils spiked with 100 mg/kg of glyphosate. Glyphosate in soil of 7, 14, 21, 28 days after spiking of glyphosate based herbicide was analyzed by spectrophotometer (Jan et al., 2009). Also soil microbial community structure was investigated using phospholipid fatty acids (PLFAs) composition analysis of soils following the procedures given by the Sherlock Microbial Identification System (MIDI Inc., Newark, DE). Glyphosate (100mg/kg soil) has no effects on reproduction and survival of *P. kimi* in any soils. Also, glyphosate in soils with Collembola was more rapidly degraded. Rapid increase of soil microbial biomass(PLFAs) was shown in soil with Collembola addition. This result showed that glyphosate affected interactions between Collembola and soil microorganisms, and also soil microbial community affected by Collembola changed degradation of glyphosate.

MO160

The environmental fate of 14C-POEA in water-sediment systems

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Tallow amine polyethoxylate, commonly referred to as POEA (polyoxyethylene alkyl amine), is a nonionic surfactant contained in many glyphosate-based formulations to improve uptake and efficacy of the active ingredient, thereby reducing the amount needed for effective weed control. A regulatory-guideline aerobic aquatic degradation study was initiated in 2012 to obtain data for potential use in ecological risk assessments for formulations containing POEA. ^{14}C-Labeled POEA was synthesized for use in the study to aid in analysis of the surfactant, to assess mineralization to CO_2, and to allow for mass balance determinations. POEA dissipated rapidly from the water column with a DT_{50} of 2 to 3 hours through a combination of metabolism and adsorption to sediment. Metabolism was extensive as evidenced by the formation of $^{14}CO_2$ ranging from 10 to 15% in the two water-sediment systems. Metabolites in the water and sediment were identified using LC-MS and confirmed using a derivatization approach. Two main classes were identified that consist of tertiary amine metabolites containing both polyoxyethylene groups on nitrogen and terminally carboxylated aliphatic chains and secondary bis-polyoxyethylene amine metabolites. Based on results of the study, along with available toxicity data, the acute and chronic risk to pelagic and benthic organisms are considered to be low due to rapid dissipation/degradation of POEA and limited bioavailability. The identified degradates, which are more polar than POEA, are also expected to pose limited exposure risk to organisms in water-sediment settings.

MO161

Biodegradation testing of chemicals with high Henry's constants - separating mass and effective concentration reveals higher rate constants

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During simulation-type biodegradation tests, volatile chemicals will continuously partition between water phase and headspace. This study addressed how (1) this partitioning affects biodegradation test results and (2) it can be accounted for by combining mass balance and dynamic biodegradation models. An aqueous mixture of 9 (semi)volatile chemicals was first prepared using passive dosing and then diluted with environmental surface water to produce test systems containing concentrations in the ng/L to μg/L range. After incubation for 2 hours to 4 weeks, automated Headspace Solid Phase Microextraction (HS-SPME) was applied directly on the test systems to measure substrate depletion by biodegradation relative to abiotic controls. HS-SPME was also applied to determine air to water partitioning ratios. Water phase biodegradation rate constants, k_{water}, were up to 72 times higher than test system biodegradation rate constants, k_{system}. True water phase degradation rate constants facilitate extrapolation to other air-water systems and are more suitable input parameters for aquatic exposure and fate models. As such, they should be considered more appropriate for risk assessments than test system rate constants.

MO162

Concentration and mixture effects on biodegradation kinetics of petroleum hydrocarbons in surface water

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Biodegradation tests are often performed at high concentrations of individual test substances, whereas environmental biodegradation is taking place at lower concentrations with many chemicals present in a mixture. Recent developments in analytical chemistry and passive dosing allow biodegradation testing of controlled mixtures at environmentally relevant concentrations. The hypothesis of this work was therefore that biodegradation kinetics of hydrocarbons in composed mixtures at environmentally relevant low concentrations generally will be similar or faster than biodegradation kinetics for single compounds tested at high concentrations. 2-Methylnonane, 1,2,4-Trimethylbenzene, and trans-Decalin were chosen as model compounds, which were studied individually and in mixtures of 3, 8, and 16 petroleum hydrocarbons. Two of them were tested individually in several concentrations. One surface water sample from a pristine brook was used as inoculum for all tests. Passive dosing from a silicone rod was used to generate stock solutions, which allowed for independent control of concentration level and mixture composition in the tests. Biodegradation experiments were conducted in 20 mL vials with 5 mL headspace. The test setup was closely aligned with the analytical method; sampling was done with automated Head Space Solid Phase Micro Extraction directly in the test vials, and coupled with Gas Chromatography – Mass Spectrometry for chemical analysis. The first results support a reduction of test substance concentrations in biodegradation testing and provide a basis for further study of the mixture effects on biodegradation.

Poly- and perfluoroalkyl substances (PFASs): Recent developments, sources, transport, fate and toxicity (P)

MO163

Quantification of sub ppt levels of per- and polyfluoroalkyl substances in various waters utilizing advanced ultra-high performance liquid chromatography and triple quadrupole mass spectrometry

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A method for the determination of per- and polyfluoroalkyl substances (PFASs) in various water matrices has been developed on the Agilent 6460, 6470 and 6495 ultra-high performance liquid chromatography coupled to triple quadrupole mass spectrometry (LC-MS/MS). Samples are prepared using solid phase extraction, either on-line or off-line. The analytical suite contains 26 PFASs including 4 perfluoroalkane sulfonates (PFSAs) (PFBS, PFHxS, PFOS, PFDS), 13 perfluoroalkyl carboxylates (PFCAs) (PFBA, PFPeA, PFHxA, PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTriDA, PFTeDA, PFHxDA, PFOcDA), 3 perfluorooctane sulfonamides (FOSAs) (FOSA, MeFOSA, EtFOSA), 2 perfluorooctane sulfonamidoethanols (FOSEs) (MeFOSE, EtFOSE), 3 perfluorooctane sulfonamidoacetic acids (FOSAAs) (FOSAA, MeFOSAA, EtFOSAA) and 6:2 fluorotelomer carboxylate (6:2 FTSA). The range of application for this method in water is 0.05-100 ng/L. Method detection limits are down to 0.05 ng/L depending on the extraction method and instrument models used.

MO164

Development and validation of a gas chromatography coupled in mass spectrometry in tandem method for the detection of fluorotelomer carboxylic acids in environmental samples

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Keywords: Fluortelomer carboxylic acids (FTCAs); environmental samples; GC-MS/MS. **Abstract** A great effort has been carried out to assess the occurrence of poly- and perfluoroalkyl substances (PFASs) in the environment. However, some congeners have been much less studied as fluorotelomer carboxylic acids (FTCAs). In spite of only some few studies have been carried out the results have proved their presence in different environmental matrices and biota. Most of the analysis methods to determine FTCAs in environmental samples are based on liquid chromatography coupled to mass spectrometry or tandem mass spectrometry approaches (LC-MS or LC-MS/MS). However, the physic-chemical properties (small molecular weight and polar character) of FTCAs makes more suitable analytical methods based on gas-chromatography coupled to mass spectrometry in tandem (GC-MS/MS) with a strong derivatization step. The main aim of this work was to develop and validate a simple, sensitive and selective new analytical methodology to determine three FTCAs (6:2 fluorotelomer carboxylic acid, 8:2 fluorotelomer carboxylic acid and 10:2 fluorotelomer carboxylic acid) based on GC-MS/MS for the detection of these compounds in different environmental matrices (water, sediments and fish). The sample pre-treatment was based on alkaline digestion for fish and ultrasonic assistant extraction for sediments. The solid phase extraction was used for water extraction and pre-concentration as well as clean-up step for fish and sediments. Finally, the samples were derivatized with BF_3-MeOH (14%) and analyzed by GC-MS/MS. The chromatographic separation was carried out using TRACE TR-5M column by TRACETM Ultra gas chromatogram (ThermoFisher Scientific). The detection was carried out with a coupled triple quadrupole mass spectrometer TSQ QuantumTM Access Max (ThermoFisher Scientific) using electro impact ionization (EI) mode in positive conditions. This new methodology offers better mLODs and mLOQs for FTCAs than the analysis by LC-MS/MS. mLODs and mLOQs are, respectively, 0.2 and 1.7 ng l^{-1} for water, 50 ng g^{-1} and 250 ng g^{-1} for fish, 50 ng g^{-1} and 250 ng g^{-1} for sediments. Most recoveries are between 60-110 % for all the matrices and compounds.

MO166

Transformation of Polyfluorinated substances in natural waters by advanced oxidation processes

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Due to their unique chemical properties, poly- and perfluoroalkyl substances (PFAS) have been historically used in several commercial applications like non-stick cookware, fire-fighting foam, and anti-stain coating. Some of these compounds are known to be bioaccumulative and persistent in the environment and have been detected in both source and finished drinking waters. Several studies have shown that there is no or relatively little removal during conventional water treatment processes. Recent studies have suggested that exposure to some per fluoro carboxylic acids, including perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), may have health implications in humans. Therefore, PFOA, PFNA and PFOS amoung others have been phased out, and regulated in the EU, US and other countries. However, with the reduction in usage of PFOA and other PFCAs, several alternative polyfluorinated compounds such as fluorotelomer acids and di-substituted polyfluoroalkyl phosphate esters (Di-PAPs) have been introduced on the market. In this study, we investigated the fate of 6:2 and 8:2 homologues of the fluorotelomer unsaturated carboxylic acids (FTUCAs) during advanced oxidation and UV treatment using ground and surface waters. Formation of PFCAs (C4 to C9) was monitored using an LC/MS-MS. Results show, the 6:2 FTUCA transformed into the 6-C PFCA (PFHxA) with a yield of 30-45% in both waters while the 8:2 FTUCA was converted to PFOA with a yield of 60- 80% during ozonation. There were minute amounts of other PFCAs formed and this suggests that an n:2 FTUCA chiefly degrades to an n-C PFCA on ozone oxidation in water. A mass balance calculation revealed that a significant fraction of parent compounds were unaccounted for in the transformation, high resolution LC/QTOF MS was then used for elucidation of additional transformation products and data will be presented. Similar trends were seen with UV-hydrogen peroxide treatment but the yields of PFCAs formed were lower With the increased use of ozone and UV-hydrogen peroxide AOP in water treatment, especially in potable water reuse, these data suggest that increased concentrations of PFCAs may result in finished drinking water.

MO167

Non-invasive Samples as a Biomarker of Human Bio-monitoring to Perfluoroalkyl substances (PFASs)

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Perfluoroalkyl substances (PFASs), which are one of the persistent organic pollutants (POPs) were known as a likely human carcinogen by the US Environmental Protection Agency's Science Advisory Boards and potential endocrine disruptor. Many researches have been examined the PFASs exposure in human and among human samples, blood has been used as an ideal bio-marker in many researches. However, collection of blood is very difficult because its method is invasive and negative perception. Thus recently, there have been attempts to assess PFASs in the human body using a non-invasive human sample including urine and hair. These kinds of samples have many advantages such as they are easier to collect, transport, store and etc. than blood. Also some previous researches have been revealed the PFASs exposure in non-invasive samples and it is indicated that non-invasive samples might be used as bio-marker for PFASs exposure in human body. Therefore, the aim of this study was examination of PFASs in same people's hair, urine and blood and finding the applicability of bio-monitoring using urine and hair instead of blood for PFCs monitoring through comparison between their concentrations. Individual serum, hair, and urine samples were collected from total 94 healthy people and eleven PFASs were investigated. Solid-phase extraction for serum and hair samples and liquid-liquid extraction for urine samples were used for extraction of PFASs and all samples were analyzed using liquid chromatography and tandem mass spectrometry (LC-MS/MS). Of the eleven PFASs, nine compounds were observed in serum, urine and hair samples. The average concentration of detected PFASs was ranged from 0.128 to 4.77 ng/mL in serum, 0.0558 to 2.24 ng/mL in urine, and 0.284 to 3.92 ng/g in hair samples. When compared each PFAS concentrations in serum, urine and hair samples to confirm the applicability of bio-monitoring using urine and hair, only PFOA showed negative correlation between serum and hair ($p < 0.05$). Above this, there is no statistically significant correlation between matrixes ($p > 0.05$), but tendency of negative correlation of several PFASs between serum and hair was shown. The detailed results will be explained in the poster.

MO168

PFAS-based Fire-Fighting Foams: a wolf in sheep's clothing?

V. BOITEUX, C. BACH, C. ROSIN, J. MUNOZ, X. DAUCHY, ANSES

Fire-fighting foams (FFFs) have been used since the 1960s to extinguish fuel-based fires (i.e., gasoline and kerosene). They are used during incidents, such as large storage tank fires and aircraft crashes, but also during regular training exercises. Fluorinated surfactants are ingredients of some of these foams. They create a thin film that spreads and floats on the fuel surface which substantially improves the fire extinguishing efficiency of the foam. This creates an effective fuel vapor barrier that puts out the fire. The foam minimizes the evaporation of flammable solvents and the fluorinated surfactants help it to completely cover the fuel surface and remain there, thus preventing re-ignition. During the present study, we analyzed nine fire-fighting foam concentrates and tried to highlight the per- and polyfluoroalkyl substances (PFASs) used in their formulations. In addition, we collected water samples in the vicinity of three sites where FFFs are or were intensively used: an oil storage depot where a large explosion occurred in 1987, a military airport and a training center for firefighters. Several fluorotelomers (FTs) were identified in the nine fire-fighting foam concentrates, however, since no standard solution was available for most of them, only a few were quantified. Some concentrates contained several PFASs, suggesting that a formulation is a complex mixture. The presence of some PFASs may be unintentional, existing as byproducts of oxidation, impurities, or unreacted starting materials. In the groundwater of the three sites, high PFAS concentrations were measured, leading to the closure of some wells used for drinking water supply. Receiving rivers were also contaminated via surface runoff or wastewater. Each site had a particular PFAS profile, due to several parameters, such as the history of the fire-training activities, the FFFs used, and the way of environmental contamination (seepage, runoff or direct discharge). When the oxidative conversion method was performed, all the PFASs were not thoroughly identified. The absence of chemical standards hinders their identification and quantification, and thereby makes it difficult to understand the fate and behavior of PFAS in the vicinity of sites where fire-fighting foams have been spilled.

MO169

Distribution of perfluorinated compounds (PFAAs) in great tits (Parus major) along a pollution gradient in Antwerp, Belgium, and their effects on reproduction.

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Perfluorinated compounds (PFAAs) have been produced for over five decades. Due to their hydrophobic and lipophobic character they are suitable for a wide range of applications. However, PFAAs may enter the environment, accumulate in wildlife and may cause detrimental effects. The widespread use of PFAAs has resulted in a global presence. Therefore the major global manufacturer, 3M, phased out the production of perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) in 2002. Nevertheless, these compounds are still detected in high concentrations in the environment and biota. Besides that, the toxicity of environmentally realistic concentrations on terrestrial songbirds is not well documented. In the present study we measured the concentration of 12 PFAAs (8 perfluoroalkyl carboxylic acids (PFCAs) and 4 perfluoroalkyl sulfonic acids (PFSAs) in non-destructive samples of a terrestrial songbird, the great tit, at a fluorochemical plant in Antwerp, Belgium. In addition, samples from four other areas were collected, representing a gradient in distance from the pollution source. At each of these locations eggs have been collected and analyzed on PFAAs. Furthermore, reproductive success at each area has been assessed and related to PFAAs concentrations in these eggs. The PFOS and perfluorodecane sulfonate (PFDS) concentrations measured at the site of the fluorochemical plant are among the highest ever reported in wildlife with median concentrations of 34251 ng/g for PFOS and 81 ng/g for PFDS. Even though PFCA levels are also among the highest reported in wildlife, levels have increased since the last study in 2011. Furthermore, these concentrations decrease sharply with distance from the plant, but remain high compared to what has been reported in literature. No effects of PFAAs have been observed on reproductive success parameters (i.e. days to first egg, clutch size, hatching success, survival, number of fledglings, fledging success and nest failure). The outcome of the present study can be used for further monitoring studies, to investigate temporal changes of PFAAs concentrations using non-destructive samples.

MO170

Degradation of Perfluorinated Alkyl Acids by Enzyme-catalyzed Oxidative Humification Reactions

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Perfluorinated alkyl acids (PFAAs) have drawn increasing concern due to their global presence, extreme persistency and environmental toxicity. The unique molecular structure of PFAAs makes them extremely resistant to degradation, and thus it is a significant challenge to remediate soil or waters contaminated by PFAAs with environmentally friendly approaches. We have conducted a series of experiments to examine the degradation of PFAAs in enzyme-catalyzed oxidative humification reactions (ECOHR) in both aqueous phase and soil slurries. The experiments with aqueous solutions were conducted using laccase as a model humification enzyme, 1-hydroxybenzotriazole (HBT) as a model mediator, and perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) as model target chemicals. We have also conducted experiments in soil slurries with laccase as the enzyme and soybean meal as supplemental natural mediators. The results of our experiments demonstrated that PFAAs can be decomposed in ECOHR under environmentally relevant conditions in both aqueous phase and soil slurries. The findings suggest that ECOHR may be effective to degrade PFAAs in the natural environment, and provide a novel scheme for in situ remediation of PFAA contamination.

MO171

Implementing a physiologically based toxicokinetic (PBTK) model describing the bioaccumulation of three perfluorinated substances in rainbow trout (Onchorynchus mykiss)

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Polyfluoroalkyl and perfluoroalkyl substances (PFASs) are ubiquitous in the environment, in particular in aquatic systems. Their unique physicochemical properties, leading to their stability, give them a complex hazard profile. PFASs behave differently compared to "classical" organic compounds and the mechanisms of absorption, distribution and elimination (ADE) remain incompletely understood, especially in aquatic vertebrates in which some of them are bioaccumulated. The aim of the present study is to describe the fundamental processes involved in ADE mechanisms in rainbow trout (*Oncorhynchus mykiss*) exposed to three model PFASs (perfluorooctane sulfonic acid - PFOS, perfluorohexane sulfonic acid – PFHxS, and perfluorononanoic acid - PFNA) through their diet, using a physiologically based toxicokinetic (PBTK) model. Also, this model will take into account the growth of individuals during their exposure. To develop our PBTK model, bioaccumulation and tissue distribution data are collected by feeding rainbow trout with contaminated pellets for 28 days, followed by a depuration period. Since temperature influences several physiological processes, the experiments will be carried out at three different temperatures (8°C, 12°C and 18°C). This poster will show the first results of this project obtained until now and will illustrate the progression of the present PBTK model.

MO172

Oxidative and metabolic effects of PFOS and its replacement F-53B after in ovo exposure of domestic chicken (Gallus gallus domesticus)

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In 2002, perfluorooctane sulfonic acid (PFOS) underwent a voluntary phase-out by its major manufacturer, 3M, after it was consistently shown to have persistent, bioaccumulative and toxic properties. Subsequently, in 2009 the production and use of PFOS was restricted by the Stockholm Convention. In China, PFOS is still being produced and used in industrial applications, e.g. as a mist suppressant in chrome-plating industry. It is expected that due to the reduction in the use of PFOS, the demand for fluorinated alternatives will increase. One of those alternatives, F-53B, has been used for decades in electroplating industry and its use might expand, also to other industries that are currently using PFOS. The structure of F-53B is closely related to that of its predecessor, PFOS. This raises concern for similar toxic effects and bioaccumulation in the environment. To our knowledge, toxicity data on F-53B are very scarce and non-existent for birds. Therefore, this study aimed to investigate the developmental toxicity of F-53B in birds, relative to and in combination with PFOS. To investigate the toxicity of these two compounds on birds, an *in ovo* experiment was performed with chicken (*Gallus gallus domesticus*) as a model species. In total, 160 chicken eggs were injected in the yolk sac with PFOS, F-53B or a mixture of both compounds. The concentrations of the compounds at single exposure were 150 and 1500 ng/g egg. For the mixtures, either compounds had the same concentrations or the low and high concentration was combined. To inject the compounds, a *vehiculum* of water and peanut oil, emulsified with lecithin, was used. Eggs were then incubated for 21 days at 37.5-38 °C and 50-70 % relative humidity. In addition, three control groups were used, i.e.

eggs injected with a *vehiculum* only, eggs with needle insertion only and eggs without injection. Shortly after hatching, chicks were decapitated and the liver was dissected and snap-frozen for later chemical and effects analysis. Gene expression of anti-oxidant responses (catalase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase), biotransformation (cytochrome P4501A) and lipid homeostasis (peroxisome proliferator-activated receptor) were analysed. The results of the gene expression analysis will be presented at the conference.

MO173

Perfluorohexanoic Acid Pharmacokinetics in Mouse, Rat, Microminipig, Pig, Monkey and Human

R.C. Buck, S. Gannon, The Chemours Company / Fluoroproducts

Perfluorohexanoic acid (PFHxA) is a perfluorocarboxylic acid (PFCA) that has been widely identified in the aqueous environment. In order to conduct a human health risk characterization, it is necessary to understand both its toxicology and phamacokinetics in mammals. The elimination kinetics of PFHxA have been determined from published data for multiple mammalian species: mouse, rat, microminipig, pig, monkey and human. For each species, the data was compiled and modeled to identify the kinetic profile of elimination from blood including calculation of elimination rate (hr-1) and elimination half-life (hr). The study shows that PFHxA elimination kinetics in mammals is consistent with a rapid initial (alpha) phase followed by a slower terminal (beta) phase. Further, the PFHxA alpha phase elimination has a first-order half-life range of 1-2 hours in mouse, rat, microminipig, pig, monkey and human. The alpha phase elimination in mammals accounts for over 99.7% of the total PFHxA elimination in mammals. PFHx-elimination is extensive in the rapid alpha phase and there are no significant pharmacokinetic differences across mammals: mice, rats, monkeys, pigs and humans. The overall conclusion of this kinetic assessment is that PFHxA does not appear to exhibit significantly different elimination kinetics across a wide range of mammalian species. The results suggest that for human health risk assessment, the interspecies pharmacokinetics adjustment factor applied to a hazard assessment benchmark should be small. Details of the assessment for each species will be presented.

MO174

6:2 Fluorotelomer Alcohol: Hazard Assessment, Human Exposure Assessment and Risk Characterization

R.C. Buck, D. Lander, S. Gannon, The Chemours Company / Fluoroproducts

6:2 Fluorotelomer alcohol is a raw material used to make surfactant and polymeric products. It has been detected in air. This paper summarizes the results of a mammalian toxicology hazard assessment and compares the results to reported exposure concentrations to characterize potential human exposure risk. The key mammalian toxicology studies are a 90-day repeated-dose oral gavage sub-chronic study in rats and a 28-day repeated dose inhalation study also in rats. In the inhalation study, no specific route of entry toxicity was observed. Consistent with systemic effects seen in the oral study were changes in body weight and effects on the teeth and the liver. The scientific results therefore support route-to-route extrapolation. Benchmark Dose Analysis (BMD) was conducted on the subchronic toxicity endpoints to determine the most sensitive effect and the corresponding BMD associated with this effect. Based on this analysis, the corresponding human equivalent dose (HED) was calculated and therefrom a human equivalent concentration (HEC) in air. This HEC was then divided by the reported indoor and outdoor air concentrations to arrive at a margin of exposure (MOE). The results show high margins of exposure (MOEs) for 6:2 FTOH inhalation exposure at reported ambient air concentrations.

MO175

The Evolution of Durable Water Repellent Technology

R.C. Buck, The Chemours Company / Fluoroproducts

Durable water repellent (DWR) functional finishes have been employed for decades to render textiles impenetrable to water (aka water repellent) and to prevent water-borne staining. Their chemical technology has evolved significantly beginning with hydrocarbon waxes and over time progressing to sophisticated high performance fluorinated finishes that deliver both oil and water repellency and stain management properties. In addition to providing essential performance demanded by consumers, DWR finishes extend the lifetime of consumer products and reduce their environmental footprint. The commercial exit of historic long-chain fluorinated DWR products has spurred the introduction of new finishes. The demand for sustainable products and chemistry by brands and consumers has raised new questions that must be considered when selecting an alternative DWR finish. Moreover, the evolution to preferred chemistry lists (aka best available technologies) curated by third party assessments is accelerating. This poster will discuss how these important trends have framed the criteria for selecting a suitable DWR finish and what the current new technologies are.

MO176

New Concawe / NICOLE technical report on the environmental fate and effects of PFAS, remedial options and legislative developments

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A recent report commissioned by the European Petroleum Refiners research division (Concawe) and the Network for Industrially Contaminated Land in Europe (NICOLE) brings together published data on the environmental fate and effects of poly- and perfluoroalkyl substances (PFAS), of which perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) are the most well-known. The unique surface tension lowering properties of PFAS have led to their use in a wide range of domestic and commercial products including high-performance class B fire-fighting foams, which are used to prevent and contain flammable liquid fires at industrial sites and airports. Emissions of PFAS may therefore arise from landfills, urban wastewater treatment facilities and fire-fighting facilities at airports, military bases and large industrial facilities. PFOS and PFOA have been identified as PBT (persistent, bioaccumulative and toxic) for humans and wildlife, which has led to low regulatory limits for these compounds. Moreover, as knowledge of the class of PFAS substances increases, restrictions are being extended to other PFAS compounds that have the potential to degrade to form PFOS, PFOA or other long-chain PFAS. Current remedial options for PFAS in soil and groundwater, including excavation and treatment with activated carbon, are resource intensive hence research is needed to develop more cost-effective, sustainable, solutions. In addition to PFAS environmental fate and effects, the report provides a concise overview of global regulatory controls, chemical analysis methods and remedial techniques to support effective, risk-based, decision making. While the report has been produced to support the work of Concawe and NICOLE members, it is anticipated that the document will be a useful source of information to others working in this area.

MO177

Effectiveness of Voluntary Programs at Controlling Emissions of Perfluoroalkyl Substances

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Increasing attention has been focused on releases of long chain perfluoroalkyl substances (long chain PFASs) into the environment, and how those releases can be controlled. The recently-concluded “2010/2015 PFOA Stewardship Program” overseen by the U.S. Environmental Protection Agency (USEPA) provides a valuable case study on the use of voluntary measures to control emissions of PFASs. The results of this program demonstrate that voluntary measures can be quite effective at removing long chain PFASs from the market and in achieving dramatic reductions in environmental exposures. Indeed, experience with the 2010/2015 PFOA Stewardship Program suggests that voluntary programs may be able to achieve meaningful reductions more quickly and efficiently than more prescriptive regulatory approaches, such as those under TSCA, REACH and the Stockholm POPs Convention.

Future challenges in sediment toxicity testing for environmental risk assessment (P)

MO178

Impact of sediment-spiked fludioxonil on nematodes - Comparison of single-species and community responses

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Lower tier studies in risk assessment of pesticides are based on single-species toxicity data. Even if using standardized laboratory toxicity tests with realistic exposure scenarios (e.g. whole sediment toxicity testing), there is still uncertainty, if obtained environmental quality standards (EQS) or regulatory acceptable concentrations (RACs) are protective for *in-situ* communities. Studies in model ecosystems (i.e. microcosm studies), can help to bridge the gap between single-species tests and field studies, providing controlled experimental conditions, and, concurrently, maximum realistic exposure, considering multiple species settings and food web interactions. In the present study, we assessed the toxicity of the fungicidal pesticide fludioxonil (FDO) in sediment-spiked microcosms. Due to its low water-solubility (OW = 4.1), FDO is expected to strongly bind to organic particles and accumulate in sediments. Therefore, we focused on the sediment inhabiting benthic fauna. In fine sediment, where highest FDO concentrations are expected, meiofaunal organisms are predominant, comprising the most abundant and species rich taxa in these habitats, such as nematodes. Effects of FDO were assessed using the toxicity test with *Caenorhabditis elegans* (nematoda; ISO 10872:2010), in aqueous and spiked sediment exposure. Moreover, nematode communities, sampled from outdoor microcoms with FDO-spiked sediments, were analyzed in terms of their abundance, species composition and NemaSPEAR[%]-index. In water, FDO showed a chronic EC50 of 0.85 mg l^{-1} for the reproduction of *C. elegans*, which is comparable to the acute toxicity for *Daphnia magna* (LC50: 1.1 mg l^{-1}). In spiked artificial and microcosm sediment, however, no inhibitory effect on *C. elegans*’ reproduction occurred up to 14580

μg/g OC, indicating a low bioavailability of FDO for *C. elegans* in spiked sediment testing. However, nematode communities responded more sensitive to FDO in sediment-spiked microcosm, with significantly lower total abundances already at 4860 μg/g OC. Therefore, standard settings based on laboratory toxicity tests with the nematode *C. elegans* have to be regarded with caution, in terms of their protectiveness for nematode communities.

MO179

Comparing the chemical and ecotoxicological persistence of sediment associated lufenuron

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Lufenuron is a benzoylurea insecticide that targets chitin synthesis in arthropods. Once entering the aquatic environment, lufenuron quickly partitions to the sediment and persists there due to its hydrophobic nature. Although lufenuron shows a limited rate of degradation in sediments, its bioavailability may decrease in time due to ageing processes. Therefore the aim of the present project was to study the impact of ageing on the ecotoxicity of sediment associated lufenuron. To this purpose, three batches of wet sediment, spiked with lufenuron concentrations (0 – 135 µg a.s. / g OC in dry sediment) were stored for zero weeks (control), five weeks and eight weeks. Subsequently these three batches of lufenuron spiked sediments were subjected to semi-chronic toxicity tests (10d) with the non-biting midge *Chironomus riparius* as well as to chemical analysis of the lufenuron concentrations. Our results showed that the lufenuron concentrations in the sediment did not decrease over time. Measured concentrations were comparable (84 – 108%) to the nominal concentrations for all ageing conditions and treatments. Consequently, the chemical half-life of lufenuron in the sediment used was substantially longer than eight weeks. Survival of *C. riparius* declined with increasing lufenuron concentrations under all ageing conditions. Two experiments yielded significantly different LC_{50}-values of 8.9 (5.4 – 12.4) and 42.5 (22.02 – 62.8) µg a.s. / g OC for sediment aged for 0w (control) and 8w, respectively. For 5w the LC_{50} could not be accurately determined due to the scattered response data. Based on the chemical analysis of the sediment, in the present study lufenuron was characterized as a persistent compound. Nevertheless, lufenuron showed a pronounced decrease in bioavailability in the sediment due to ageing. This was indicated by the five-fold increase of the LC_{50}-value for lufenuron after ageing lufenuron-spiked sediment for 8w. Comparing the chemical and ecotoxicological persistence of sediment associated lufenuron thus showed that even for very persistent compounds adverse environmental effects may diminish over time, although these compounds may remain present in the environment for a very long time.

MO180

Practices and challenges in toxicity testing of mining affected fresh water sediments.

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Mining increases metal concentrations in receiving water bodies. Part of these metals will sink to sediments and may induce toxic effects. There is a limited selection for standardized ecotoxicological tests for sediments and even those may be problematic in the case of multi-metal contamination. We studied four mining affected lake sediments in Finland plus a reference sediment. The sites were analyzed for metal content (ICP-MS), SEM-AVS (simultaneously extracted metals – acid volatile sulfides), and water chemistry. Sediment toxicity was tested with luminescence bacteria (*Vibrio fischeri*, ISO), sediment-dwelling oligochaete (*Lumbriculus variegatus*, OECD) and non-biting midge (*Chironomus riparius*, OECD). In addition, the usability of standard snail test (*Lymnaea stagnalis*, OECD) to sediments was tested. Metal concentrations in all of the study lakes were high, especially for Pb, Mn, Zn, Ni, Cu. Also, the ?SEM were systematically higher than in the reference lake. Luminescence bacteria test did not give reliable results, since the EC_{50} values between bulk sediment and sediment pore waters had more than 1 000 -fold variation. All the other used toxicity testing methods had problems with drastically dropping pH. For *L. variegatus*, the sediments with lowest metal content were the most toxic. These were the sediments with lowest pH at the end of the experiment and the highest organic matter content. The most toxic sediments were from the lakes with the softest waters. With *L. variegatus*, the pH problem was partly bypassed by not aerating the test chambers. The oxygen levels stayed in the acceptable levels (> 30%) even without aeration. For *C. riparius*, the required oxygen level (> 60%) was not reached without aeration and they did not survive the low pH. *L. stagnalis* was not as sensitive to pH changes as other organisms. These experiments show the difficulties when standard toxicity tests are used for metal-contaminated sediments. Besides metals, water chemistry and other substances (e.g. SO_4, DOC and particle size) were seen to influence the results.

MO181

Formulated metal-spiked freshwater sediments with different metal bioavailability for toxicity and bioaccumulation tests

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Whole-sediment tests with metal-spiked sediments are often used to assess the toxicity and the bioaccumulation potential of metals in benthic invertebrates. Formulated sediments are considered too simplified for these studies because they lack solid-phase ligands, such as organic carbon (OC), Fe and Mn oxide minerals and acid volatile sulfide (AVS), which affect metal availability to biota. For this reason natural sediments spiked with metals are favored for whole-sediment toxicity testing. However, the wide variation in their physico-chemical characteristics, besides the solid-phase ligands, makes often difficult to establish a clear relationship between total concentrations of metals in sediments and their toxicity to benthic organisms. Variability of master parameters such as pH and conductivity may also act as confounding factors. In our preliminary study, we aimed to obtain standard sediments with diverse metal bioavailability by varying their contents in solid-phase ligands. To this purpose, we investigated the feasibility to prepare such metal-spiked formulated sediments and followed metal partitioning in the different matrices with ageing. Five sediments were prepared: one formulated sediment according to OECD guidelines and four variants of this sediment with different amounts of OC, AVS, Fe and Mn . Each sediment was spiked with the same concentrations of Ni, Cr and Co according to the 2-step spiking method. Sediments were aged for 70 days in semi-static conditions with OECD formulated water. In order to avoid sediment perturbation during the sampling, the sediment was aliquoted into individual 50-mL vials and put into a plastic aquaria filled with formulated water. Overlying water (OW) was monitored through time for master parameters (pH, conductivity, hardness, oxygenation and alkalinity), at day 1, 2 and 6 during the first week, and then once a week until 10 sampling points were reached. No significant differences were detected between the five scenarios: pH was quite constant through time whereas conductivity and hardness fall down in the first days and reached a steady-state after 10 days. For each scenario, metal concentrations (Ni, Cr, Co, Fe and Mn) were monitoring in OW, pore water and sediment sampled, as well as OC and AVS contents in sediment.

MO182

OCCURRENCE OF POLYCYCLIC AROMATIC HYDROCARBONS IN THE SEDIMENTS OF THE UMGENI AND MSUNDUZI RIVERS IN KWAZULU-NATAL PROVINCE, SOUTH AFRICA

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Polycyclic aromatic hydrocarbons (PAHs) can exist in over 100 different combinations of fused aromatic rings. They occur ubiquitously in environmental matrices worldwide. They are persistent organic pollutants (POPs) that are lipophilic in nature. Most are formed as a result of incomplete combustion of carbon-containing materials, such as oil, wood, cigarettes, garbage or coal, and also from other activities, such as driving, agricultural burning, paving with asphalt, road construction and so on. Some PAHs have been observed to be toxic, carcinogenic, mutagenic and teratogenic and those not found to be carcinogenic may act as synergists. This prompted an investigation into the presence of the 16 EPA PAHs in sediment samples of the uMgeni and Msunduzi rivers in KwaZulu-Natal Province. Little or no information is available on the concentrations and distribution of PAHs in these two KwaZulu-Natal rivers. These two rivers have anthropogenic, agricultural and industrial activities which make them an appropriate site of study. There are dams and tributaries along the courses of these rivers including various effluent discharge points from waste treatment plants. Eighteen sediment samples were collected along the courses of the two rivers for the summer season. These sediment samples were dried and partitioned into different mesh sizes with a sieve. Extraction of the sediments was carried out with the aid of an ultrasonic bath and dichloromethane as the extracting solvent. The extracts were cleaned by using silica gel, anhydrous sodium sulfate and dichloromethane as eluting solvent. The almost dry purified extracts were made up to 1 mL with dichloromethane and transferred to GC vials with the addition of the internal standard. Quality assurance and quality control measures were observed. The presence of these PAHs in the sediment samples was determined by means of gas chromatography-mass spectrometry (GC-MS). The results obtained indicate that the 16 EPA PAHs are present in the sediment samples. The method exhibited good recoveries of 80 to 118% and the percentage relative standard deviations were observed to be < 5%.

MO183

SETAC Sediments Interest Group

T.L. Sorell, Brown and Caldwell / Risk and Toxicology

Hazard and risk assessment of human pharmaceuticals in the

environment (P)

MO184

Integrated ecotoxicological and epidemiological approach to evaluate the impact of Tamiflu metabolites on reproduction of medaka (Oryzias latipes)

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The purpose of this study is assessing the effects of environmental relevant Tamiflu metabolite concentration on long-term survival, growth and reproduction of Taiwan medaka (*Oryzias latipes*). Firstly, this study to predict the environmental Tamiflu metabolites concentration from the perspective of age-specific influenza infected population and treatment dosages by epidemiological susceptible-infectious-recovered modeling. Second, wecarried out the 56 days long-term toxicity experiments with medaka varying with predicted environmental relevant Tamiflu metabolite concentrations to observe survival and growth energy budget. Finally, we carried out the reproductive bioassays to characterize the egg production and hatchability and to identify suitable biomarker. Simulated results showed that the predicted environmental Tamiflu metabolite concentrations from influenza outbreak of type B, A(H1N1), and A(H3N2) were 12. 82 (2.25 – 48.88), 17.95 (2.26 – 25.37), and 47.03 (2.42 – 134.44) μg L^{-1}, respectively based on 2014 and 2015 influenza confirmed cases in Taiwan. Hence, this study selected 0.06, 0.3 90, and 300 μg L^{-1} as environmental relevant Tamiflu metabolite exposure concentration to process bioassays. Experimental results showed that both survival and daily growth rate were not significant differences between control and exposure groups. However, the egg production of 0.3 and 300 μg L^{-1} were significantly decreased those compared to control group, whereas the hatchability had a downward trend in exposure group of 300 μg L^{-1}. These results indicated that environmental relevant Tamiflu metabolites did not affected survival and growth energy budget of medaka. However, if the pandemic influenza outbreak induced higher than 300 μg L^{-1} Tamiflu metabolites, it could be a potential hazard for medaka. Otherwise, this study suggests that hatchability is a suitable biomarker to monitor ecological health. This study provided the insights into the ecological risk assessments under seasonal and pandemic influenza outbreak.

MO185

THE POTENTIAL EFFECTS OF ENVIRONMENTAL LEVELS OF THE HIV DRUG NEVIRAPINE ON THE BEHAVIOUR, GROWTH AND DEVELOPMENT OF EARLY LIFE STAGES OF THE MOZAMBIQUE TILAPIA (Oreochromis mossambicus)

U. Nibamureke, I. Wagenaar, University of Johannesburg / Zoology; I. Barnhoorn, University of Venda / Zoology

South Africa is believed to be one of the leading countries in HIV cases in the world, it is also the country with the highest number of people on HIV anti-retroviral (ARVs) therapy. Over the last three years, ARVs have been detected throughout South African surface and drinking waters. Nevirapine is one of the first-line HIV ARVs which is prescribed to newborn, infants, children and pregnant women to prevent mother to child HIV-1 transmission. The effects of ARVs on fish health and their reproduction is unknown. The aim of this study was to assess the potential effects of nevirapine, on the growth and development of early life stages of the freshwater fish *Oreochromis mossambicus* through a chronic exposure. The early life stages of *O. mossambicus* including yolk sac embryos and free swimming larvae were exposed to environmental relevant concentrations of nevirapine (1, 48 μg/L) in a static renewal system in a controlled environment (26 ± 1°C; 14:10 day/night cycle) for 30 days. The endpoints that were assessed include feeding, swimming and schooling behaviour, survival, growth and development (length and weight). A histo-morphological assessment was also done on whole individual samples (1, 5, 30 days old) to assess any microscopic changes on internal tissues and organs. Preliminary results show that the environmental levels of nevirapine in South African surface waters have no significant macroscopic effects to the behaviour, survival, growth and development of yolk sac embryos and free swimming larvae of *Oreochromis mossambicus*. As nevirapine has proved to be important in controlling the HIV infection from mothers to children, these results will contribute in understanding its fate in the aquatic environment and will help to plan and recommend enhanced waste water treatment methods.

MO186

Evaluation of a metabolomics-based fish embryo assay for predicting drug induced long-term effects in fish

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Within the IMI project iPiE (Intelligence-led Assessment of Pharmaceuticals in the Environment), a more sophisticated environmental testing and tools for prioritization of legacy compounds shall be developed. Regarding evaluation of fish toxicity, screening approaches in fish embryos are pursued allowing faster assessment and taking into account 3R aspects. While the standard fish embryo toxicity (FET) test is restricted to lethal parameters more relevant as substitutes for acute effects, additional sub- lethal endpoints may provide expanded applicability of the FET assay for chronic effect assessment in fish. In this respect, the analysis of the metabolome could yield insights into biochemical processes elicited by pharmaceutical compounds and could potentially support the extrapolation from fish embryo to chronic fish toxicity as displayed in the standard early life stage (ELS) test. In this context, a pilot study was performed with the aim to quantify and comparatively assess changes in the metabolic signatures of fish and fish embryos induced by the reference compound amikacin, an aminoglycoside antibiotic, in order to identify metabolic patterns applicable as biomarker profiles that can be linked to apical endpoints, such as survival and growth. Therefore, toxic effects in fathead minnow embryos and ELS fish were investigated following a 4 and 32 days exposure, respectively, examining conventional endpoints and additionally using a targeted assay for metabolic profiling in whole body extracts of ELS fish and fish embryos. Metabolic endpoints were found to be at least as sensitive as standard apical endpoints in both individual study types. Furthermore, multivariate data analysis revealed amikacin induced metabolic perturbations specific for fish and fish embryos as well as shared marker candidates, which were consistent with expected metabolite changes of aminoglycoside induced renal toxicity in mammals. Further studies using compounds with different pharmacological activities are ongoing to provide a more in-depth investigation of the suitability of metabolomics techniques as a prediction tool for chronic fish toxicity and mode-of-action markers in fathead minnows. This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (iPiE grant n° 115735)

MO187

Environmental risk assessment of active pharmaceutical ingredients used in human medicinal products: Europe-wide variation in risk quotient.

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In accordance with Article 8(3) of Directive 2001/83/EC, as amended, a new marketing authorisation application shall be accompanied by the evaluation of the potential environmental risks posed by the human medicinal product. These environmental risk assessments (ERAs) estimate the potential environmental impact on a product-by-product basis rather than a substance-by-substance basis. In the cases where an active pharmaceutical ingredient (API), or substance, is used in multiple therapeutic indications, or products, there is the potential to under estimate the environmental risks. To determine the total substance or API risk, independent of therapeutic use, we have (i) collated definitive predicted no effect concentrations (PNECs) for over 150 APIs (excluding anti-infectives), (ii) collated human consumption data for each of these APIs in European Countries where these products are licenced for use, (iii) conducted a worst case exposure assessment and estimate the total risk posed by each API, (iv) analysed the variability in the risk quotients (RQs) for each API across the EU. This presentation will summarise these analyses and demonstrate that, using these worst case assumptions, (i) most APIs (>90%) pose little environment risk, (ii) the highest RQs are associated with endocrine active APIs, and (iii) RQs for the same API can vary by 2-3 orders of magnitude between EU Member States.

MO188

pH-Dependence of Bacterial Toxicity: Role of Speciation for Ionisable Pharmaceuticals

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Active pharmaceutical ingredients (APIs) are designed to be biologically active and may adversely affect non-target organisms in the environment with acute and chronic effects. To estimate the toxicity of chemicals quantitative structure activity relationships (QSARs) have been developed, which are based on physicochemical properties of the compound. A measure of the specificity of the effect of a compound is the toxic ratio (TR), which is the quotient of the effect concentration calculated with a baseline-toxicity QSAR and the experimental effect concentration. The TR provides guidance whether a compound acts according to baseline toxicity (TR< 10) or a reactive or specific mode of toxic action (TR≥10). Established baseline toxicity QSAR models are mostly based on the octanol-water partition coefficient K_{ow}, simply by a linear relationship of log EC_{50} and log K_{ow}. The domain of applicability of most baseline toxicity QSARs comprises neutral organic chemicals ranging from log K_{ow} of approximately 1 to 7. About 70-80% of all APIs are ionisable and are therefore not part of the applicability domain. The pH can affect their chemical speciation, i.e. the fraction of charged and uncharged forms, and this can result in a different toxicity. Thus, the role of speciation has to be considered for toxicity QSAR development, e.g. by using the pH-dependent liposome water distribution ratio D_{lipw}(pH). In this study we used the bioluminescence inhibition test with *Aliivibrio fischeri* (Microtox). APIs can be considered as baseline toxicants in the bacterium *A. fischeri*, with the exception of antibiotics. Compounds that act as uncouplers which directly interfere with the energy production, since the bioluminescence is dependent on ATP, are also specifically acting. Here we aimed to test the hypothesis if by replacing K_{ow} or D_{ow}(pH) in baseline toxicity QSARs by the D_{lipw}(pH) many APIs will be classified as baseline toxicants. We investigated the pH dependence of cytotoxicity between pH 5.5 and 9. Most APIs fell on the range of baseline toxicity when ionization-corrected membrane-water partition coefficients D_{lipw} (pH) are used as descriptor. We observed a pH dependent toxicity for ionisable APIs, which suggests that the neutral species is the driver of toxicity. This supports the use of the D_{lipw} (pH) as physicochemical descriptor in baseline-toxicity QSARs for other bioassays to identify APIs with specific mode of action indicated by a TR>10.

MO189

Factors influencing freshwater pharmaceutical uptake using in vitro gill cell culture

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Pharmaceuticals are becoming increasingly prevalent in the environment and regulations to assess the environmental risks requires the use of fish in animal testing. A primary fish gill cell culture system has shown significant promise as an *in vitro* replacement model system for whole fish compound uptake studies. The current study assessed the uptake of 8 pharmaceuticals with a range of chemical parameters from the apical freshwater compartment of the primary gill cell culture system. An HPLC method was developed to extract and analyze compounds simultaneously from the OECD water matrix (warfarin, ibuprofen, beclomethasone, carbamazepine, diclofenac, ketoprofen, norethindrone, gemfibrozil). The relationship between the uptake of the compounds and the various chemical parameters were analysed using linear regression. There was no correlation to Log K_{ow} (R=0.002), weaker correlation to Log S (R^2=0.24) and molecular weight (R^2=0.23), and a stronger correlation to Log D (R^2=0.57), and pKa (R^2=0.56). The strength of correlation of Log S (R^2=0.83) and pKa (R^2=0.91) were greatly improved with carbamazepine and diclofenac excluded, respectively. Uptake of the compounds was found to be a function of cell permeability and overall findings suggest that ionizable pharmaceuticals are able to interact and enter fish gill cells.

MO190

Development of a zebrafish embryo test for the evaluation of endocrine disrupting pharmaceuticals with nano-injection as an alternative exposure route

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Pharmaceutical companies have to perform an environmental risk assessment for every new drug. The tests for potential endocrine disrupting (ED) compounds require a lot of time and test animals, which is not consistent with the 3R principle. Therefore, the goal of this study is to develop a zebrafish embryo test, which is not considered an animal test according to European regulations. However, it is often difficult to expose fish aquatically to ED pharmaceuticals because of their lipophilicity. Nano-injection is therefore proposed as an alternative exposure route because the yolk of zebrafish embryos contains many lipids, and this route mimics maternal transfer. To use nano-injection as an alternative it needs to be characterised and compared to the classical exposure route via water, because it is known that toxicity can depend on the exposure route. In this part of the study 17α-ethinylestradiol (EE2, estrogen receptor (ER) agonist) was chosen to compare malformations, swimming activity, ER activation and uptake dynamics between both routes. Transgenic (*5xERE-GFP*, obtained from the Carnegie Institution of Washington) zebrafish embryos, expressing GFP upon ER activation or wildtype embryos were aquatically exposed or injected and observed until 120 hours post fertilization (hpf). 0.5 nl of EE2 dissolved in dimethyl sulfoxide (DMSO) was injected. DMSO was also used as vehicle control. Each day, fluorescent intensity was measured to compare the time-dependent dynamics of ER activation in both routes and malformations were scored. Growth was monitored daily from 54 hpf and swimming activity from 72 hpf. We observed different physiological effects and malformations between both routes. During the 5 days, fluorescent signals were in the same order of magnitude for both routes. This indicates that EE2 was still active 5 days after injection. The fluorescent intensity increased every day in both exposure scenarios, similar to the increase of *esr2b* mRNA levels during normal development. However, the relative contributions of EE2 accumulation and ER expression to the signal are not known yet. The dynamics of ER activation are similar between both routes, suggesting that other mechanisms (e.g. uptake in different organs) are responsible for the morphological and physiological differences. This future knowledge will be essential to determine the applicability of nano-injection as an exposure route for lipophilic compounds.

MO191

Calcium channel blockers and antihistamines: specific and non-specific toxicity in Daphnia magna

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Human pharmaceuticals that were authorized for a specific indication before 2006 are exempt from the requirement of a formal environmental risk assessment. Yet, various approaches have been proposed to prioritize these biologically active legacy compounds for an assessment of their potential effects in the environment. Within the project "iPiE" (*Intelligence-led Assessment of Pharmaceuticals in the Environment*, supported by Europe's Innovative Medicines Initiative (IMI)) such a prioritization scheme will be developed that utilizes also model-predicted properties for compounds without available data. Yet, only few data on human pharmaceuticals are available for developing models for acute and chronic *Daphnia* toxicity. Therefore, a series of acute and chronic *Daphnia magna* tests were conducted according to OECD Guidelines 202 and 211 with pharmaceuticals from two therapeutic groups (calcium channel blockers and antihistamines). The individual test compounds covered a range of physico-chemical properties, particularly with regard to lipophilicity and ionization. Correlations between physico-chemical properties and observed effects on survival, reproduction and growth of *Daphnia magna* will help to identify key descriptors for models of non-specific toxicity and patterns that point at specific modes of toxicity in *Daphnia*.

MO192

Impact of pharmaceuticals on aquatic invertebrates: an indoor study

I. Roessink, Alterra / Environmental Risk Assessment Team; E. Peeters, Wageningen University / Aquatic Ecology and Water Quality Management

Nowadays pharmaceuticals are measured frequently in surface water monitoring schemes. These substances reach surface waters via waste water treatment plants (WWTP) and are distributed throughout the receiving water system. The effect these substances might have on the aquatic environment, however, is in most cases not known. To study the potential effects of pharmaceuticals, several laboratory experiments were conducted varying from single species behavioural assays to indoor microcosm (using a small ecosystem) testing. In the laboratory, 16 microcosms were installed containing a small aquatic community comprising *Elodea* sp. (plant), *Lumbriculus variegatus* (worm), *Physella* sp. (snail), *Asellus aquaticus* (macrocrustacean) and *Daphnia magna* (cladoceran). Four microcosms were filled with 12L effluent, four with 12L control water and four were filled with 12L control water and received a spike of a mix of selected pharmaceuticals. The mix comprised metformin, guanylurea, metoprolol, sotalol, atenolol, irbersartan, hydrochlorothiazide, diclofenac and carbamazepine. In addition, single species behavioural assays were conducted using the Multispecies Freshwater Biomonitor (MFB) exposing *Gammarus pulex* (macrocrustacean) to fluoxetine, ibuprofen, carbamazepine and CTAB. In the microcosms the exposure to pharmaceuticals at effluent relevant concentrations did not cause effects on the survival on the tested populations of *Lumbriculus*, *Physella*, *Asellus* and *Daphnia*. The excess of nutrients from the effluent seems actually to favour the populations, resulting in increases in abundance compared to the control. In contrast, MFB assays with *Gammarus*, another macrocrustacean, showed that at low concentrations effect on behaviour were observed which again disappeared at higher test concentrations. These results indicate that pharmaceuticals do not follow the standard (eco)toxicological rules where an increase on concentration shows and increase in effects but have much more subtle effect windows. Such effects, however, will be difficult to observe in model ecosystems where only a part of the aquatic community is present.

MO193

Fate of pharmaceuticals in surface waters; a case study of the river Dommel, The Netherlands

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Nowadays pharmaceuticals are measured frequently in surface water monitoring schemes. Their distribution in water and sediment depends on various parameters, e.g., substance characteristics, degradation and sorption kinetics. These parameters, however, are usually not known frustrating predictions on their environmental fate. In this project a DUFLOW model was parameterized to model the distribution of diclofenac, metoprolol, carbamazepine and sulfamethoxazole (SMX) in the river Dommel, The Netherlands. The model was validated using available monitoring data. Parameterization of the model was performed using tailored laboratory experiments, surface water and sediment monitoring data. In the laboratory set-up the impact of temperature, photolysis and microbial activity on compound degradation was tested. The data on surface water concentrations was obtained from regional surface water quality surveys, while sediment data was obtained by sampling and analysing sediment from different locations in the Dommel system. In the lab study, diclofenac degraded fastest (DT_{50}=2.8d), followed by SMX (DT_{50}=8.3d), metoprolol (DT_{50}=10.6d) and carbamazepine (DT_{50}=22.2d). Photolysis, increased temperature, and microbial activity all enhanced compound degradation. Although modelled dynamics of carbamazepine and metoprolol fitted the measured concentrations well, this was not the case for diclofenac. Since SMX was not included in the analysis of the monitoring surveys, the modelled dynamics could not be validated against measured concentrations in water and sediment. The DUFLOW model predicted the dynamics of metoprolol and carbamazepine well but did not so for diclofenac which was the most degradable compound tested. A sensitivity analysis on the different model parameters, i.e., degradation, resuspension, sedimentation rate and partitioning coefficients, revealed that model sensitivity was high towards degradation rates of the compounds. Since the laboratory study showed that degradation was driven for a large extend by photolysis, this indicates that good knowledges of this process and its confounding factors (e.g. DOC, DIC, algae Chl-a) is key to adequately model the fate dynamics of fast degradable pharmaceuticals in water systems. For slower degrading compounds the current model already provides adequate fits with measured pharmaceutical levels.

MO194

Long-term effects of the antiepileptic drug carbamazepine in Chironomus riparius - two multi-generation studies

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Midges like *Chironomus riparius* are mobile organisms, which can either be exposed to chemicals continuously for several generations or populate uncontaminated habitats. Especially for contaminants like the anti-epileptic drug carbamazepine (CBZ) that is emitted throughout the entire year, the investigation of multi-generational effects is necessary to assess its risk in the environment. In the current study we aim to investigate i) whether the response to long-term exposure changes within three subsequent generations (F_0, F_1, F_2) and ii) whether future unexposed generations are affected by the pre-exposure of the parental generation (F_0). Chironomid larvae (< 24 h old) were exposed to nominal sediment concentrations of 3.2 and 6.4 mg/kg CBZ (measured time-weighted means of 0.68 and 1.66 mg/kg, respectively) in F_0. Hatched larvae of the following generation were transferred into two separate experiments. In the continuous exposure experiment, *C. riparius* was exposed for two subsequent generations (F_1 and F_2) and to the same concentrations as in F_0. In parallel, a recovery experiment was conducted, where larvae were transferred into unexposed medium in F_1 and F_2. As endpoints, mortality and mean emergence time of males and females were investigated. At a concentration of 3.2 mg/kg continuously exposed midges reached control level within three subsequent generations. Midges were successfully selected for reduced sensitivity to CBZ. In addition, pre-exposed midges recovered with the absence of the stressor. At the higher exposure level of 6.4 mg/kg, the sensitivity of the midges first decreased in F_1 before mortality increased again in F_2. As these results were similar for pre-exposed midges that were transferred in unexposed medium, selection of less sensitive midges in F_1 and inbreeding depression due to the reduction of the gene pool could explain the observations. Especially for mobile organisms like midges a combination of continuous exposure and recovery is a realistic test scenario. Multi-generational experiments showed to be a helpful tool to investigate long-term effects of a chemical on midges.
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MO195

The environmental concentration of medicinal chemicals for human use in urban rivers in Japan

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Chemicals used as the active ingredients in medicines discharge into the aquatic environment. Although the amount of each chemical might be minute, it has been feared affect derived by the chemical properties and physiological properties to the organism. However, we still know little about what kind of impact on wildlife in the environment. In view of these facts, we have measured the actual environmental concentrations of 31 kinds of active ingredients in marketing medicine, using liquid-chromatography with mass spectrometry in representative seven urban rivers in Japan, fall and winter seasons in 2015-2016. The maximum detected concentrations of the active ingredients exceeded 100ng/L are shown below,olmesartan (571ng/L), valsartan (405ng/L), irbesartan (162ng/L), candesartan (113ng/L), losartan (117ng/L) for antihypertensive agent, and sulpiride (546ng/L) for antipsychotic, cralythromycin (445ng/L) for antibacterial agent, ketoprofen (150ng/L) for analgesic antipyretic, bezafibrate (200ng/L) for hyperlipidemia treatment drug, crotamiton (845ng/L) for antipruritic. Among target ingredients, the detection concentration of active ingredient in medicines for the lifestyle-related diseases tended to be higher. The cconcentration in the winter was observed a higher tendency when compared in the fall and winter. It can be assumed that the flow rate of the river is related. It must be considered that five active ingredients used in the same disease, antihypertensive agents, were detected in high concentrations, because ingredients detected at high concentration might be discharged in the same amount every day. In the future, it would need to be evaluated in consideration the combined action of multiple active ingredients based on the mechanism of action. We have been established the environmental impact assessment guidance about the active ingredients of medical supply that put out newly in the market. Environmental Risk Assessment is carried out by comparing Predicted Environmental Concentration (PEC) and Predictive Non-Effective Concentration obtained from the biological tests followed the method which the EMA proposes. In 31 active ingredients targeted in this study, the measured detection concentration was less than the PEC value except for two active ingredients, olmesartan and rosvastatin. This research is supported by the Research on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and development, AMED.

MO196

Sorption and degradation potential of pharmaceuticals in stormwater ponds receiving wastewater contributions

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Pharmaceuticals can enter aquatic environment through urban stormwater runoff as it contains not only pollutants from land surfaces, but also wastewater contributions from combined sewer overflows and sewage misconnected to storm drains. A large part of the urban stormwater runoff is treated in retention ponds prior to discharge to receiving waters. These ponds offer conditions that can affect pharmaceuticals, but multiple physicochemical and biological properties of the compounds make their fate rather complex to predict. In the present study, we look into sorption and degradation of selected pharmaceuticals by sediments from stormwater retention ponds that knowingly receive misconnected wastewater. The core of the study is a microcosm investigation where we study the fate of biocides under conditions mimicking real stormwater ponds. We substantiate the microcosm results by determining the potential for biological degradation under oxidizing and reducing conditions, and by determining how sorption depends on pH. Five pharmaceuticals are selected: carbamazepine (CAR), sulfamethoxazole (SUL), naproxen (NAP), furosemide (FUR) and fenofibrate (FEN). The microcosms hold a sediment bed and a pond water phase to which the pharmaceuticals are added and their fate monitored in both water phase and sediment. Prior to addition of pharmaceuticals and at termination of the experiments, the sediment beds are depth-profiled for dissolved oxygen, redox potential and pH. Together with the determined degradation and sorption potentials, this information is used to understand the fate of the pharmaceuticals in the microcosms. Initial experiments indicated that adsorption to un-buffered sediments fitted a Langmuir model with distribution coefficients K_d of 3.5, 3.7, 3.8, 4.4, 571 mL/g for NAP, CAR, SUL, FUR and FEN, respectively. In other words, the first 4 compounds were more mobile in the water phase. Initial tests with the microcosms showed that there is a clear gradient in both redox potential and pH in the sediment beds. Hence the dependency of sorption on pH must be taken into account when understanding the fate of pharmaceuticals in stormwater ponds, as must the biological degradation under oxidizing versus reducing conditions. The suggested poster will present the experiments in their completeness. Results from this study can give a clearer understanding of how stormwater retention ponds affect selected pharmaceuticals prior to discharge into the aquatic environment.

MO197

Development of threshold of ecotoxicological concerns (eco-TTC) for assessing the environmental safety of industrial chemicals and human pharmaceuticals

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In the absence of chemical specific toxicity data, the threshold of toxicological concern (TTC) approach provides a method to manage threshold of human exposure below which “there would be no appreciable risk to human health”. The approach attempt to determine a level of use of chemicals which could be considered to be safe, regardless of its degree of toxicity. TTC has been developed to assess chemicals in foods (flavorings and food contact materials) and pharmaceutical genotoxic impurities. Due to the practical applicability of the approach in regulatory context, development of the reliable TTC approach is expected for assessing the environmental safety of chemical substances and pharmaceuticals with *de minimis* exposure. In this study, we analyzed the ecotoxicity test data sets of 400 industrial chemicals assessed by Japanese Ministry of Environment and 70 human pharmaceuticals published by European Medical Agency. All the toxicity studies have been performed in compliance with the standard OECD test guidelines and Good Laboratory Practice. Taking a probabilistic view, the 5th percentile of the distribution of NOEC is assigned as a threshold value for toxicity. For industrial chemicals and pharmaceuticals respectively, TTC values of 0.025 and 0.007 mg/L were derived from NOEC values for growth inhibition test of algae at 72 hours. And threshold values of 0.063 and 0.010 mg/L were obtained from NOEC values for *Daphnia magna* reproduction test at 21 days. Our preliminary analysis indicated that some substances with pyrene, acrylate, urea or carbamate substructure have lower PNEC values than the threshold values for growth inhibition of algae. Some anilines, phenols, phosphorothioates or steroids were found to have lower PNEC values than the TTC values for effect on reproductive output of *Daphnia magna*. The results suggest that these chemical groups are possible exclusions for use of TTC approach. However, further analysis will be needed with increased number of data set of human pharmaceuticals and fish chronic toxicity endpoint. This work was supported by the Research on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and Development (AMED).

MO198

Human health risk assessment of pharmaceuticals and other emerging contaminants in river Kokemäenjoki and artificial ground water in Finland

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Today a number of pharmaceuticals and other emerging and traditional chemicals can be measured from surface and ground water all over the world due to enhanced chemical techniques. However, the relevance for health risk assessment for many of these chemicals is still either unknown or unproved. Acute health effects of chemicals in Finnish surface waters are rare due to low concentrations and long term effects especially for emerging contaminants is not yet to be found in many cases. A wide range of chemicals was measured in river Kokemäenjoki in 2012-2015 in surface water and in artificial ground water production site that uses pre-treated river water as raw water. Selected emerging contaminants, including pharmaceuticals and artificial sweeteners, and perfluoroalkyl acids (PFAA) were measured from water samples with an isotope dilution mass spectrometry (IDMS) method . Human exposure and health risk assessment was calculated for the chemicals in artificial ground water to estimate the overall health risk of contaminants in tap water in Turku region in Finland. In first phase the exposure level of chemicals was determined. Secondly the health risks were estimated by comparing the exposure levels to acceptable daily intake (ADI) levels. Thirdly, the number of illness cases was calculated when applicable for health effect of pharmaceuticals and other chemicals proven to cause human health effects in long term epidemiological studies. The studied compounds were detected in river water in concentrations of 0.05 - 1740 ng/L. Highest concentrations were measured for an artificial sweetener acesulfame. In exposure assessment daily intake of contaminants via orally consumed tap water was estimated and inhabitants were exposed for ibuprofein, acesulfame and caffeine in notable amounts via drinking. Nevertheless when comparing to the chemicals' ADI values, none of the chemicals exceeded the ADI values therefore proving that the contaminants measured in the artificial ground water do not cause any harm to human health in current regulatory perspective. However, some of the chemicals such as some PFAAs are proved to be harmful to human health in long term exposure and those effects were investigated in more detail in this study to demonstrate the need for more complex risk assessment for chemicals in surface and ground water used for drinking water production.

MO199
Hazard assessment of human pharmaceuticals - a review of data on persistence, bioaccumulation, and toxicity
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Hazard assessment of persistence, bioaccumulation and toxicity is a central part of environmental risk assessment (ERA) in legislations such as for biocides, pesticides and pharmaceuticals. Hazardous substances classified as persistent, bioaccumulative and toxic (PBT) or very persistent and very bioaccumulative (vPvB) are of very high concern, as they are highly toxic and can accumulate in the in the environment as well as in the food web. Therefore, no save environmental concentration can be defined and the entry of PBT substances into the environment should be minimized. This poster presents a review of hazard assessment data of active pharmaceutical ingredients (APIs) received over the last 10 years of authorization of human pharmaceutical products (HMPs) in accordance with the 'Guideline on the environmental risk assessment of medicinal products for human use' (EMEA/CHMP/SWP/4447/00, rev. 2) describing the procedure and data requirements for ERA of HMPs. Assessment of persistence of APIs is generally based on standard OECD tests on transformation in water and sediment system (OECD 308). Analysis of 152 studies according to OECD 308 revealed that more than 60% of APIs are classified as persistent or very persistent in one or more compartments. Among those, about 20% of APIs are persistent or very persistent in the water phase. Regarding bioaccumulation, analysis of 49 studies on bioconcentration in fish (according to OECD305) illustrated that about 20% of APIs are classified as bioaccumulative in relation to the PBT criteria (bioconcentration factor, BCF>2000). Among those APIs, more than half show a logKow below 4.5. This is in line with the requirement for data on bioconcentration in fish for APIs with a logKow >3 (EMEA/CHMP/SWP/4447/00 corr 2). The toxicity criterion is fulfilled if chronic toxicity (NOEC/LOEC) is below 0.01 mg/L or, the substance is classified as CMR (carcinogenic, mutagenic or reprotoxic). So far, the review revealed that eight APIs were classified as PBT therefore display a hazard. For that reason, monitoring and management strategies are needed to minimize the entry of these hazardous APIs into the environment. In conclusion, this review illustrates that more than 60% of the investigated APIs are persistent in the environment. However, for many of the persistent APIs reviewed, it was not possible to finalize the PBT assessment due to incomplete data sets. PBT assessment is important to identify APIs of very high concern.

MO200
Soil enzymes as biomarkers of pharmaceutical effluents' toxicity
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Components of many pharmaceuticals have characteristics that may cause them to be regarded as dangerous wastes when disposed into the environment (soil or water bodies). In other to ascertain the toxicity of these pharmaceuticals in the environment, we monitored their effects, *in situ*, on the activities of some soil enzymes. The soil enzymes which are known to play significant roles in nutrient cycling and ecosystem stability, phosphatases, dehydrogenases and ureases, were used as markers or biomornitoring agents for toxicity. Dehydrogenases and phosphatase activities as well as urease activity were determined according to standard spectrophotometric methods. Enzyme activities in soil samples spiked with other pharmaceuticals were also determined for correlation.Resultsrevealed that the effluents had negative effect through the enzyme activity. Significant changes ($p < 0.05$) in activities of these soil enzymes relative to control, are indications of toxicity of the pharmaceutical effluents. This was collaborated with soil samples spiked with pharmaceuticals. The correlation showed that as urease activity increased, dehydrogenase and phosphatase activities decreased. It becomes apparent that some components of the pharmaceutical effluent may have a deregulatory effect on the soil microbial load or have direct inhibition on the enzymes which are involved in mineral/nutrient cycling in the soil.

MO201
From PEC-based trigger to a reasonable decision tree: Revison of the Phase I in the environmental risk assessment of human pharmaceuticals
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The "Guideline on the Environmental risk Assessment of Medical Products for Human Use" (EMA/CHMP/SWP/4447/00 corr 2) came into effect on 01. December 2006. The phased approach consist of a pre-screening phase (Phase I) to estimate the exposure of the pharmaceutical compounds to surface waters. Based on the action limit of 0.01 μg/L a risk assessment is required in the second phase (Phase II) and base set of aquatic toxicology and fate data need to be provided. Based on ten years of experience in assessing the environmental risk for human pharmaceuticals a new "pre-assessment" scheme is proposed. It is structured as a decision tree mainly to identify pharmaceutical compounds, which do not require an in depth ERA incl. ERA studies. For these compounds, only a justification for the absence of an in depth ERA is required. There are two suggested key elements, which characterise the proposed "pre-assessment" phase: the abolishment of the 0.01 μg/L - action limit for Phase II an ERA is not needed anymore for orphan drugs The poster presents a first draft of a new "pre-assessment" decision tree consisting of six questions. The questions are covering legislative issues, orphan status, pharmacokinetic properties and exemptions due to the nature of the active pharmaceutical substance. The aim is a transparent and simple approach to decide if an environmental risk assessment is needed and for which active moiety. For this purpose, clear definitions and criteria are needed besides comprehensible explanations of the used questions. This newly proposed approach may be considered in the ongoing revision of the current EMA guideline. Furthermore, this "pre-assessment" scheme may also be used in prioritisation approaches for the identification of environmentally relevant pharmaceutical substances.

MO202
Non-clinical toxicological data and environmental risk assessment - A regulatory point of view
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Human pharmaceuticals are extensively studied before approval and use in humans. Toxicological and pharmacological properties in mammal models have to be assessed as well as the environmental risk of the active pharmaceutical ingredients (APIs). One read across hypothesis states that an API, usually because of structural similarity and conservation, is likely to exhibit similar properties or follows a regular pattern with similar modes of action (MOA) across diverse taxa. There is increasing interest in using mammalian non-clinical data to extrapolate to wildlife species for estimation of environmental effects and further assessment of the risk posed by API exposition to the environment. However, less work has been conducted on direct comparison of non-clinical and ecotoxicological data from specific APIs out of their application dossiers. The purpose of this study was to evaluate whether, or rather to what extent, available non-clinical toxicological data from various API can be related to ecotoxicological endpoints. First, the pharmaceutical development process was analysed for potential toxicological and ecotoxicological information concerning the API. We put the focus on the MOA in mammals and its possible reference to wildlife species. Application dossiers available to the German Environment Agency (from 2005 to 2016) were screened with regard to the submitted non-clinical toxicological and ecotoxicological data. Non-clinical toxicological study design depends on the indication and is individual for each AIP. As comprehensive data are available for chronic diseases, e.g. diabetes, rheumatism or cardiovascular diseases, our study focused on these

indications. We compared specific studies, MOAs as well as endpoints and discussed the evaluation from a regulatory point of view. The analysis is still ongoing and will be presented in detail.

MO203

Environmental risk assessment of human pharmaceuticals in the European Union: A review of the estimation of environmental exposure

E. Nfon, Smithers Viscient

There has been a marked increase in the last few years in concern regarding the fate and effect of pharmaceuticals in the environment following the detection of pharmaceuticals in sewage treatment plant (STP) effluents, surface waters, groundwater and even drinking water. The current regulation of pharmaceuticals requires an environmental risk assessment as part of a marketing authorisation application in the EU and a new drug application in the US. Guidance issued by the EMA and the FDA describes how the pharmaceutical active substances should be assessed for possible adverse effects in the environment. In both regimes a simple approach is recommended to estimate the exposure concentration. These estimation methods generally average the exposure concentration in the environment that is treated as a homogenous entity. However the work of the FOCUS Working group clearly demonstrates that the EU is a diversity of environments hence spatially explicit models that take into account the specific characteristics of the drainage basin are more relevant, especially for pharmaceuticals that enter the environment solely as a result of human use. Two such models that are already in common use are: PhATE™ (Pharmaceutical Assessment and Transport Evaluation) model and the Geo-referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER). PhATE is commonly used in the US and the exposure estimates predicted by PhATE™ generally had a good fit with measured data from the USGS. While GREAT-ER has already been used in the exposure modelling of pharmaceuticals in European surface waters, it is not part of the environmental risk assessment of human pharmaceuticals in the EU. Many regions are already moving towards a watershed/catchment based water quality management since it is recognized that a thorough understanding of the potential environmental exposure is a prerequisite for a reliable assessment of risk. It is our view that the current procedure used to estimate the environmental exposure to human pharmaceuticals is overly simplistic and argue that integrating spatially explicit models would provide a basis for developing a proper strategy of the environmental risk assessment of human pharmaceuticals.

MO204

SETAC Pharmaceuticals Interest Group

M. Williams, CSIRO / Land and Water

Wastewater effluents: How research can improve risk assessment and regulation (P)

MO205

Characterization and exposure assessment of seasonal and weather dynamics in pollutant mixtures from wastewater discharge

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Sites of wastewater discharge have been generally identified as hotspots for contamination with organic micropollutants. Consequently, these sites are often associated with high risks for aquatic organisms. Wastewater treatment plant (WWTP) effluents contribute significantly to the overall flow and pollution particularly of small rivers. In order to improve monitoring, assessment and management of these hotspots, the aim of this study is to unravel the temporal dynamics in the emitted pollution mix. This characterization helps to shed light on the complexity of the effluent composition and to identify critical time windows of elevated exposure of aquatic organisms. The study was performed at the Holtemme River (Saxony-Anhalt, Germany). Effluent samples during a week in May, July, October 2015 and February 2016 were provided by a WWTP operating in a separate sewer system. Target screening by LC-MS/MS (AB Sciex QTRAP® 6500) included 155 target compounds (pharmaceuticals, pesticides and industrial and lifestyle compounds). From these compounds, 85 compounds were detected in at least one sample and 46 in all WWTP samples. Principal component analysis revealed a clear distinction of the different months based on their chemical profile. This indicates that the risk to aquatic organisms in the receiving river also varies with time. Considering the relation of within-week variation to between-week variation of each compound, 42 of 85 substances were classified as “constant” discharge - mainly pharmaceuticals, biocides and legacy pesticides - representing a baseline risk which can be complemented by individual seasonally occurring compounds. These compounds were almost entirely composed of pesticides with significant seasonal peak loads (e.g. boscalid = 5670 mg/d, MCPA = 80854 mg/d), whereas some pestcides showed a clear weekly application pattern (e.g. pethoxamide, metolachlor, terbuthylazine and its metabolite). As these compounds are applied often together, this led to the conclusion that the effluent directly reflects the agricultural activities in the surrounding area. Since this WWTP operates in a separate sewer system, storm water run-off cannot explain the input but rather incorrect disposal and cleaning practices. Further investigations including samples from the receiving river and additional sources of pollution such as associated rain sewers will complete unravelling entry routes and pollutant concentrations.

MO206

Fate of hydrocarbons and other substances in petroleum refinery waste water treatment processes

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Several directives and regulations in Europe address surface water quality or the reduction of water pollution. The main EU instrument regulating pollutant emissions from industrial installations is the Industrial Emissions Directive (IED) which aims to achieve a high level of protection of human health and the environment by reducing harmful industrial emissions across the EU. This is achieved via application of best available techniques with BAT associated emission limits (AELs) defined in the BAT reference documents (BREF) such as the BREF for the refining of mineral oil and gas. A recent CONCAWE study was carried out to characterize the relationship between effluent concentrations of a number of analytes (metals, organics) and the type of waste water treatment process. We investigated the fraction removal of contaminants such as oil in water (OiW), benzene, toluene, ethyl benzene and xylene (BTEX), polyaromatic hydrocarbons (PAH) and metals in various steps of the waste water treatment process of 14 European oil refineries. The aim was to investigate concentration shifts and relate them to specific waste water treatment plant (WWTP) processes and/or physical/chemical characteristics of the waste waters. The removal factors for OiW varied by 96% to >99% between refineries, and between treatment systems although the variation in final effluent concentrations was less. The final effluent concentrations were low and consistent within effluent limits. Certain treatment steps were able to remove specific substances, such as OiW by dissolved air flotation (DAF) system and BTEX in the biotreatment step. Additional to the contaminant analyses, toxicity tests were performed on the effluents which is discussed in another abstract. A key focus of the study was to investigate shifts in the hydrocarbon block (HCB) composition for different treatment steps as this offers a potential to link removals to reductions in toxicity. Relationships were found between the HCB removal factors and physical-chemical properties of these groups such as volatility and solubility. Insoluble constituents found in particulate oil were effectively removed with other solids. The data provided insights in the different steps of the waste water treatment and the removal of effluent parameters and hydrocarbons. This information may be useful in the context of the next BREF review and potentially provide additional data for petroleum substances REACH dossiers.

MO207

Risk assessment of Danube River pollution by pesticide residues in the vicinity of Novi Sad

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Danube River pollution in the vicinity of Novi Sad caused by the direct and indirect discharge of urban and industrial wastes and run-off has led to the accumulation of toxic compounds such as pesticides, surfactants, halogenated aromatics and hydrocarbons onto river sediments, indicating their possible uptake by biota and a potential risk for the environment. The wells of Danube River alluvion are important source of raw water for drinking water production for Novi Sad inhabitants. Therefore, screening and target analyses of selected priority, priority hazardous and emerging substances in samples of wastewater and surface water as well as raw water were performed in order to detect potential risk for the water environment. Within the sampling campaigns of Danube river water and wastewater discharged directly into Danube, organochlorine pesticides were detected in the highest concentrations, especially in the wastewater, indicating pollution from agricultural areas and activities, households and farms in the vicinity of Novi Sad. The presence of emerging and hazardous priority substances in wastewater implies the pollution of surface and raw water. The risk assessment analysis was performed based on identification of the extent of exceedance of ecotoxicity thresholds, PNEC values and data obtained from target and screening analytical results. The most accurate PNEC values were already defined in the existing EU legislation, while for other compounds they were either identified in the ecotoxicity databases or estimated by evaluation of QSAR data of certain compound for fish (Pimephales promelas), Daphnia magna and algae (Selenastrum capricornutum). The exceedance of PNEC was observed for heptachlor, heptachlor epoxide, p,p`-DDD, endosulfan-alpha, chlorpyrifos, dieldrin, hexachlorocyclohexane-gamma, hexachlorobenzene, which indicates that for those pesticides routine monitoring and the derivation of EQS values should be performed. **Acknowledgement:** The research has been supported by NATO Science for Peace Project (ESP.EAP.SFP 984087) and III46009 Project.

MO208

Persistent pollutant flux estimator tool: predicting recovery from legacy contamination in rivers

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Substances characterised as Persistent, Bioaccumulative and Toxic (PBT) have the potential to cause significant harm in the aquatic environment. Many of these substances are poorly removed in conventional Wastewater Treatment Works (WWTW) and once discharged may accumulate in downstream sediments and biota. Estimating the flux of these chemicals in receiving waters is complex due to the combined effect of accumulative and reductive factors and consequently the overall effect of controls on discharge concentrations are difficult to determine, particularly when changes occur over a timescale of many years. With the aim of assessing the risk posed by PBT chemicals a prototype calculator has been developed to estimate the flux and resulting concentrations of PBT chemicals in sediment and biota over time. The model estimates the flux as a function of the residual or base load, the rate of input and the rate of release from sediment, which provides a method for assessing the effect of a change in inputs on the concentrations of PBT chemicals in sediment and biota over a 20 year planning horizon. Following model development, we estimated the potential effect of two options for control of WWTW discharge concentrations of brominated diphenyl ethers (BDEs). BDEs were used historically as flame retardants and are classified as a Priority Hazardous Substance under the Water Framework Directive (congeners 28, 47, 99, 100, 153, 154). The simulated controls were (1) a continual year-on-year percentage reduction in inputs (e.g. an ongoing decrease in use) and (2) a step change reduction in inputs (e.g. a substance ban or the implementation of a new treatment technology). Our study found that in order to ensure adherence to the biota EQS (0.0085μg/kg), a reduction in BDE input of 37% year on year is required. In the alternative scenario (complete cessation of emissions) the concentration in the environment would take 6 (± 2) years to fall below the biota EQS. The prototype calculator may be easily adapted to estimate the effect of controls on the presence of other PBT type chemicals in the environment. Possible scenarios for other trace contaminants will be presented.

MO209

Biological effects measures and adverse outcome pathways applied to effluent assessment

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A review of the use of biological tools for effluent and mixture assessment was conducted. Whole organism tests and well defined/ validated biomarkers are both widely used in mixture assessments, with tacit acknowledgement of scientific short-comings. Adverse outcome pathways (AOPs) have been proposed as a basis for interpreting biological effect tools. As a concept, this shows considerable promise but in terms of practical or regulatory application, especially to mixtures assessment, this may be a long-term aspiration. One reason for this is that the AOP/ biomarker/ mixture approach is constrained by the sheer number of available permutations. There are, however, examples of where the AOP approach has been applied with some success, especially in situations where an effluent is well characterised and the biological effect tools are targeted to a known class of substances. The regulatory use of AOPs and biological techniques should be applied in the context of a readily identifiable data anchor (e.g. the results from more traditional whole organism bioassays). This is because with biological effects tools there is a need to extrapolate to effects at the population level and therefore there is some uncertainty in the interpretation of the outcomes from these tests and potentially more regulatory conservatism. There is also a requirement to establish clear, unequivocal baselines, and benchmarks of biological effects measures if they are to be used as more than just screens in earlier tiers of hazard and risk assessment. A potential framework is proposed that may provide a pragmatic and transparent way forward for the environmental impact assessment of industrial effluents. *Acknowledgement* - The authors thank the GICPER, gathering Companies: ARKEMA, EDF, L'OREAL, ORIL Industrie, SANOFI, SOLVAY, and TOTAL for funding this work.

MO210

Effect assessment of complex mixtures on the thyroid axis of X. laevis. A case study with reclaimed waters and carbamazepine

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Concern about the constant presence of pharmaceuticals and personal care products in surface waters, attributed mainly to the discharge of treated wastewaters from Waste Water Treatment Plants (WWTP), is renowned. The sustainable practice of using reclaimed waters for irrigation, groundwater recharge or other potential uses like aquaculture, in regions with water scarcity, could exacerbate the problem as tertiary treatments do not always remove these compounds completely. Although information about the effects of pharmaceuticals and personal care products on aquatic organisms individually is increasing, data about the effects of these compounds in complex mixtures, as naturally occurred, are limited. The main concern related the presence of these emerging contaminants is their chronic or sublethal toxicity. On the other hand, endocrine disruption at the thyroid axis level has still not been studied in-depth for many aquatic pollutants. This work aims to discuss the potential effects of using reclaimed water on the thyroid axis of *Xenopus laevis* sp. For this purpose, an AMA test with some modifications was developed by exposing *Xenopus laevis* larvae to reclaimed water, and to reclaimed water spiked with carbamazepine at concentrations 100 and 1000 higher than the mean levels observed in reclaimed waters. Carbamazepine was selected as it is considered a marker of anthropogenic pollution and could have a potential effect on thyroid axis. Morphological endpoints (e.g., wet weight, developmental stage, hind limb length, whole body length, snout to vent length), histological alterations of the thyroid gland and mRNA expression of genes related to thyroid hormones were studied. The results are discussed. This work has been funded by the Spanish Ministry of Economy, Industry and Competiveness through CTM2013-44986-R and CTM2014-52388-R.

MO211

Evaluation of toxicological effects of drugs present in wastewater obtained from Bolzano water treatment plant (WWTP) on HepG2 and HT29 cells

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A wide variety of drugs may be present in the aquatic environment (rivers, lakes and streams) at low concentrations, in particular in wastewater of urban origin. Depending on the applied treatments, such drugs may be present at very variable concentration into effluents from wastewater-treatment plant (WWTP). Such complex of substances, generally difficult to characterize, may represents a challenge for risk assessment, due to possible synergistic effects which may lead to an increase or a decrease of their biological activity. Moreover, set-up of a battery of cell-based bioassays to assess health-relevant biological endpoints may be useful to integrate chemical analysis for water quality assessment. The aim of this study was to assess efficacy of water treatment processes of WWTP of Bolzano (Italy), evaluating possible toxicological effects on HepG2 and HT29 cells due to drugs present in water samples, measuring both primary nonspecific toxicity (cytotoxicity and ROS production), as well as markers of inflammation (IL-6 production) and xenobiotic metabolism (modification of CYP1As and CYP3A4 expression). Chemical analysis of two wastewater samples revealed the presence of several drugs, including paracetamol, salbutamol, atenolol, ranitidine, sulfamethoxazole, omeprazole, carbamazepine, ketoprofen, diclofenac, clarithromycin and bezafibrate.Generally,exposure for 48-72 h to wastewater didn't affect HepG2 and HT29 cell proliferation. Even if concentration of drugs increased by 12 times that detected in wastewater samples, no toxic effects were observed. In both cell lines, ROS production was assessed after 1, 2, 3 and 24 h exposure without obtaining appreciable differences from controls. Moreover, no significant effects in IL6 production was observed in wastewater-treated cells. As regard CYPs expression, some variation was observed after treatment with wastewater samples. Interestingly, in HepG2 cell line, an increase was observed in CYP3A4 protein expression, the most expressed CYPs in human liver, after 48 h exposure, on the contrary a significant decrease after 72 h was observed. As exposure time to wastewater samples differently affects CYPs expression in HepG2 but mixture of drugs didn't affect CYPs expression, this effect is probably due to other substances in wastewater samples. In conclusion, it has been demonstrated that wastewater collected from WWTP of Bolzano shows a safe toxicological profile in HT29 and HepG2 cell lines.

MO212

Innovative ways to discern causative factors for toxic effects of refinery effluents

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As part of continuing environmental research to assess the quality of oil refinery effluents, waste water was sampled from 14 different refineries across Europe and evaluated using Whole Effluent Toxicity assessments. Acute and chronic toxicity was determined using bacteria (30 min; Microtox), algae (72hr; *Pseudokirchneriella subcapitata*), daphnids (21 days, *Daphnia magna*) and fish larvae (6 days; *Danio rerio*). As significant, albeit variable, effects were observed with all four organisms, questions arose regarding which factors caused the observed toxicity. Possible factors include metals, hydrocarbons acting via narcosis, organic toxicants with a more specific mode of action and confounding factors such as conductivity or ammonia. The work focused on developing novel approaches to discern the responsible factors in a manner which could be routinely applied to assessments of refinery effluents. As the possible toxic effects of metals and confounding factors can be relatively easily assessed by routine chemical monitoring, research focused on the organic contaminants. For 18 samples (two refineries provided three samples each) toxicity tests were performed on reconstituted samples derived on XAD-extracts which effectively removed metals and other confounding factors. Chemical analyses, including Total Petroleum Hydrocarbons (TPH) and solid phase micro extraction (SPME) were performed to estimate exposure levels and assess the levels of potential bioaccumulating substances (PBS). Bacterial and daphnia toxicity showed a good correlation with PBS, while only minimal effects were observed for algae and fish larvae. On the basis of these studies PBS measurements and/or Microtox tests seemed suitable to provide an initial indication of toxicity for compounds acting via nonpolar narcosis. Finally, effect-directed analysis (EDA) was applied to assess the possible presence of toxicants with a specific mode of action. Several samples were fractionated using liquid chromatography and each fraction assessed with Microtox and acute daphnia tests undertaken in 96-well plates. This screening method showed that compounds, others than petroleum hydrocarbons, were detected with indications that some were responsible for toxicity in several effluent samples.

MO213

Toxicity assessment of acrylamide and epichlorohydrin from combined use of polyelectrolites in waste water treatment towards Daphnia magna and Caenorhabditis elegans

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Polyelectrolites are widely used as industrial aids and flocculants in municipal and industrial waste water treatments. Also, these are used to de-water the limed sewage sludge when it is to be used as fertilizer, in order to achieve the minimum dry matter contents provided by directives.\n The flocculation process consists of two main steps: a primary flocculation, where coagulating agents are used to destabilize flocs, followed by a secondary flocculation, in which the destabilized flocs are then agglomerate by use of long-chain/high-molecular-weight polymers. The electrical charges brought by both coagulants and flocculants play an important role in the choice and combination of these.\n One of the many coagulating agents found on the market is a copolymer of epichlorohydrin-dimethylamine. Such product might bring into the wastewater flow residuals of its production that can be then released into waterways or sewage sludge, and subsequently on agricultural fields. Among the residuals, epichlorohydrin(ECH) can be of relevant importance, since it has been shown to induce genotoxic, reproductive and carcinogenic effects in in-vitro and in-vivo studies.\nFor treatment of drinking water, waste water, and dewatering of sludge, flocculants such as long-chain polyacrylamides(PAMs) are used. Acrylamide(AM) is the monomer widely used as an intermediate in the production of PAMs. Due to the low cost and broad application of PAMs, a certain amount of free AM is expected to end up in the water stream and likely sewage sludge. In fact, according to the World Health Organization, the most important source of drinking-water contamination by AM is the use of PAMs flocculants containing residual levels of AM monomer. PAMs are non-toxic, but the monomer AM is a known neurotoxin and classified as a "probable human carcinogen" by IARC.\nData on human health and environmental risk for ECH and AM have been previously presented, but the toxicity of a mixture of these two has not yet been investigated, according to our knowledge. \nIn this study, the aquatic and terrestrial toxicity will be assessed on *Daphnia magna* and *Caenorhabditis elegans*, in order to predict the likely adverse effects when ECH-copolymers and PAMs are combined in the waste water treatment with dewatering of sludge. The assumption is that the toxicity of flocculants residuals, towards fresh water and terrestrial organisms, might be of higher relevance when these are combined in a mixture, than when tested singularly.

MO214

Identification of recalcitrant toxic organics in oil refining wastewater effluents from Colombia as indicators of treatment efficiency

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Globally, the number of lethal victims from illnesses related to polluted water is higher than the number of victims of violent deaths, including war. This is the reflection of a global water quality crisis partly due to the discharge of highly contaminated industrial wastes into water bodies, which affect their composition and create a hazard potential. Industrial wastewater treatments play a key role in the release of toxic pollutants to the environment and define the pollution potential of a given sector. Within industry, the petroleum refining industry has been classified as one of the most contaminating sectors worldwide and its wastes may threaten future water security, which makes them a high-priority research field. Yet, traditional monitoring of refining wastewater does not provide information regarding composition and toxicity, which is necessary to design successful treatment technologies to impede pollutants from entering water bodies. This study characterized wastewater effluents collected in a petroleum refinery located in Colombia and identified the main toxic organic compounds, which are therefore resisting the treatment applied. Briefly, contaminants were extracted from wastewaters and then analysed using a combined approach including both chemical (separation and identification methods) and biological (bacteria-based toxicity test) techniques in order to correlate toxicity and individual contaminants. To our knowledge, this is the first study characterizing Colombian refining discharge samples, and the results could explain some of the local environmental problems involving river contamination and mass fish death. The obtained results will be used to evaluate sustainable, low-cost technologies targeting key contaminants, reducing the overall toxicity being discharged into water bodies.

MO215

Toxicological Evaluations of Effluents from Kara Cow Market, Ogun State, Nigeria on Guppy Fish (Poecilia reticulata)

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The Ogun river in Ogun state, Nigeria is a sink for untreated organic effluents from the Kara cow market. In this study, the potential toxicological effects of effluents from the Kara market were evaluated in *Poecilia reticulata* (Guppy fish). The methods used were questionnaires administration, physico-chemical and microbiological analyses of effluents from the market and histological evaluations in *P. reticulata* exposed to sublethal concentrations of the effluents over a period of 56 days. The results of the questionnaires administration showed that 50% to 90% of the respondents confirmed that the cows drink water from the river, effluents were not treated before discharge into the river and detrimental health effects have been observed due to use of the river. Physico-chemical analysis of the effluents revealed that oil and grease, heavy metals and pH levels exceeded the WHO limits. Microbiological evaluations showed the presence of coliforms, pathogens and Fungi. The median lethal concentration (96 h LC_{50}) of the effluents to *P. reticulata* was 71.50 mL/L (7.15%). Histological alterations observed include congestion of primary gill filaments and hyperplasia of secondary lamellae in the gills, hypertrophy of epithelial cells and inflammation of the intestinal microvilli in the intestine and mild congestion, degeneration of the epidermis in the skin of *P. reticulata* over the exposure period. We recommend the installation of an effluent treatment plant at the market to reduce the pollution load of the effluents discharged into the Ogun river. This will forestall potential environmental and public health epidemics to humans and other organisms that utilize the river's resources.

MO216

Impact of industrial waste water treatment plants on Dutch surface waters and drinking water impact

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Direct emissions of emerging and well known contaminants via industrial wastewater treatment plants (IWTPs) considerably impact Dutch surface water concentrations with vulnerable drinking water functions. In this nationwide impact study, the impact of 182 Dutch IWTPs was modelled, whilst taking geographic distribution, climatic variation, impact from abroad and variable types of industries into account. Modelled contaminants were 1,2-dichloroethane, DEHP, dichloromethane, benzene, toluene and vinyl chloride. Contaminant loads were estimated by normalizing emissions registered in the European Pollutant and Transfers Register (E-PRTR) over the Total Organic Carbon (TOC) emission per economic sector, and successively projecting these on actuals TOC emissions per IWTP. Only 13% of the IWTPs contribute the majority of impact on drinking water production. Between 65,6 and 80% of the average impact of all 6 modelled contaminants, on drinking water production volumes, originates from a single Dutch IWTP. Industrial sectors with the highest impact are the refined petroleum industry, paper industry and plastic industry.

MO217

Online Biomonitoring in wastewater treatment plants

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Micropollutants, such as pharmaceuticals, biocides and pesticides, can only partly be removed in traditional wastewater treatment technologies, thus several countries install advanced purification steps based on either oxidation or active coal filtration or a combination of both. Some substances, however, cannot be removed at all, degradation and transformation products might even increase toxicity, leading to a toxic cocktail difficult to evaluate and to control. In order to evaluate the success of the reduction of the toxic potential of advanced purification steps, and/or to monitor the toxic potential effluents from smaller WWTPs (without advanced steps) and/or to monitor the toxic status of WWTPs planning to build an additional step, automated online biomonitoring might provide important information on both acute toxic effects by toxic pulses and chronic toxic effects by low-dose chemicals. The biomonitor can be placed either in the effluent (emission control), above/below an advanced purification step (process control) or directly in the receiving stream (immission control). The biomonitor was operated successfully with *G. fossarum* and reached stand-alone times of up to 4 weeks. Examples from (1) effluent monitoring and (2) process control are presented.

MO218
Environmental impact and health risk assessment of urban wastewater reclamation and reuse scenarios
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Water reuse schemes are increasing as population require more clean water and better ways to manage wastewater. At the same time, water scarcity and implications about use risk of reclaimed water have get attention of population and authotities. From this starting point, the focus of this study is to determine the environmetal benefits and impacts of the reuse scheme implemented in the city of Sabadell by using Human Health Risk Assessment (HHRA), Life Cycle Assesment (LCA) and Water Impact Index (WIIX) methodologies. With this approach, the aim was to show that the reuse scheme is not a threat to public health and environment and thanks to that, to increase the confidence in the water reuse schemes for both public authorities and population. Water ready to be reused is obtained at the Sabadell wastewater treatment plant. It accounts a conventional primary treatment and an advanced secondary treatment that includes a Membrane Bioreactor (MBR) in order to produce reclaimed water. That water is used in Sabadell mainly for street cleaning, green areas irrigation and for urban uses such us toilet flushing in a commercial area. New uses are being promoted in order to supply golf courses in the region for irrigation.

Input/output and Hybrid Life Cycle Assessment for supporting the assessment of production and consumption patterns (P)

MO219
Improving market activities in the ecoinvent LCI database with GMRIO data
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1. Motivation The ecoinvent LCI database aims to cover the whole global economy. While the inventory data on a process level is very detailed, the economic model is simplistic. Industrial activities are connected by “market activities”, which are based on the production volumes of the producing activities. Apart from the electricity sector, where extensive regionalization efforts have been undertaken, market activities in ecoinvent are predominantly modelled as global markets. However, many goods aren’t traded globally and the global market composition is not representative of local conditions. Since they are based on trade analysis, global multi-regional input-output (GMRIO) databases like Exiobase can be used to improve market activities. **2. Methods** We disaggregate global market activities in the ecoinvent database into regional markets, whenever several regional producing activities are available. The composition of the newly created markets is based on the production volume within those regions (“domestic” production minus exports) and imports from the other markets of the same product. Markets are matched to economic sectors in Exiobase and the relevant economic interactions are extracted from the input-output tables. Production volumes remain consistent with ecoinvent data, only the trade information from Exiobase is added to ecoinvent. Hence, it is possible to improve the supply-chain modelling by market information from Exiobase without sacrificing level of detail in ecoinvent. **3. Results and conclusions** Biogas is an example of a product, which currently is modelled as a globally traded good in ecoinvent. The composition of the global market for biogas is approximately 50% from anaerobic digestion of sewage sludge and 50% from anaerobic digestion of manure. While, detailed process information for biogas production in Switzerland is available in ecoinvent, it is linked to the global market. However, close to 90% of the biogas produced in Switzerland originates from anaerobic digestion of sewage sludge and therefore the global market is not representative. The new improved market activities include this circumstance, which otherwise skews results for processes in Switzerland that consume biogas. Trade of biogas between the local Swiss and the rest-of-world (RoW) market is included, based on trade data from Exiobase. The new market activities have a significant potential to bring the ecoinvent database closer to the economic reality.

MO220
Uncertainty characterization in hybridised life cycle inventories - A case study on mobility scenarios in Luxembourg
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Life cycle inventory (LCI) data collection is limited by temporal and financial constraints, leading to compromises on the system boundaries and resulting in cut-offs. Producing hybridised inventories by merging the inventory data with multi-regional Environmentally Extended Input–Output (EEIO) databases, can improve the completeness of the modelled system and thus reduce this truncation error. Linking existing LCI databases and data collected from stakeholders with IO data introduces additional sources of uncertainty. This includes parameter uncertainty within the IO data and uncertainty caused by simplifications or assumptions about model structure. EEIO databases however do not provide uncertainty measures, thus hindering the inclusion of their parameter uncertainties into the commonly applied uncertainty propagation schemes for LCIs. In our case study on mobility scenarios in Luxembourg, which falls within the context of a consequential life cycle assessment (CLCA) using agent-based modelling (ABM), we are confronted with uncertainties arising from these multiple data sources. In this preliminary work we propose a methodto address the challenge of estimating the uncertainty in our hybridised LCA output. To quantify the uncertainty forthcoming from this hybridised inventory, the uncertainties of the elements in the technosphere and biosphere have to be specified. For the elements coming from the EEIO database and theforeground datanormal distributionsare assumed, with a mean given by the parameter’s value and a standard deviation of 10%. A Monte Carlo analysis is performed to propagate the parameter uncertainty forthcoming from the entire hybridised LCI. At the current stage of the project a set of mobility scenarios has been specified and a preliminary hybridised LCI has been built, while the ABM simulator is still under development. As the source system demand is planned to be derived using the ABM, we for now investigate the results of individual transportation means i.e. buses. Hybridised LCIs are argued to reduce the truncation error, yet the lack of quantitative uncertainty measures for EEIO databases still poses some concern. While our implementation of uncertainty quantification can contribute by giving a means to estimate the uncertainty in the model output based on assumptions, this can by no means replace an uncertainty analysis based on quantitative uncertainty measures.

MO221
Parametric Hybrid Life Cycle Assessment of Proton Exchange Membrane Fuel Cells (PEMFCs) for light-duty vehicles
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According to projections of International Energy Agency, to comply with the European GHGs emission target the provisioned penetration of Fuel Cell Electric Vehicles (FCEV) is estimated as the 25% of the total passenger light-duty vehicles in 2050. Therefore, a massive production of Proton Exchange Membrane Fuel Cell (PEMFC) is expected for the next decades, as well as an extensive penetration of hydrogen production facilities and distribution infrastructures. In this research, a Life Cycle Assessment model has been developed and applied to a PEMFC operating in different future scenarios given by a combination of the following parameters: geographic contexts (Japan and Germany), platinum loads (0.142, 0.3 and 0.4 mg/cm2), and hydrogen production technologies (steam reforming and electrolysis). The LCA has been performed in a cradle-to-gate approach, with the aim to account for the non-renewable energy and GHG emissions embodied in 1 kWh produced by the fuel cell in the aforementioned future scenarios. The developed LCA model is based on an Integrated Hybrid Input-Output model, which constitutes the computational structure of both Life Cycle Inventory Analysis and Impact Assessment stages. According to this approach, inventories of primary and secondary inputs required for the construction and the operation phases of the fuel cell (foreground processes) are collected based on the Authors’ experience in PEMFC technology, the in-depth literature surveys and the support of the Ecoinvent® database. On the other hand, supply chains have been modeled trough the World Input-Output Database (WIOD – background system), which provides bilateral symmetric industry-by-industry World Monetary Input-Output Tables covering all the years from 1995 to 2014. For the construction phase, it is found that the environmental impact of the fuel cell slightly depends by the geographical location, while it is strongly affected by the platinum load. On the other hand, the operation phase (i.e. the production and distribution of the hydrogen) is independent from the platinum load, while it is strongly affected by the geographical location. Due to the provisioned hydrogen production technologies assumed for the analysis, it is found that overall environmental impact of the operation phase is one order of magnitude higher than the construction phase.

MO222
Towards an assessment of biodiversity impact of financial investments
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Among the banking sector, there is a growing concern about how their investments can cause biodiversity loss. But how can a bank go from the money they spend in investments to an estimate of the biodiversity lost? We were commissioned by ASN Bank to answer this question, namely to assess the biodiversity impact of its investment portfolio. For this purpose, we have developed an approach to assess the biodiversity footprint of their investment portfolio. The approach was developed as an iterative process, including interviews and stakeholders meeting with

representatives from government, methodological experts and representatives of civil society organisations. It consists in a quantitative assessment complemented with a qualitative analysis. The quantitative assessment considers all investments made and the trade flows between countries and sectors using the Exiobase database. The ReCiPe method was used for characterising the damages caused on ecosystems. It is backed-up by a qualitative analysis to identify the general limitations of the quantitative analysis and the specific limitations to a given investment. This approach combines simple and pragmatic data collection with easy to grasp and transparent results.

MO223
Development of a regional material flow model to account for solid waste generation and management
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Although, the waste management and policy are regionalized, complete and detailed data on the generation and treatment of waste are often unavailable at regional level. For the Brussels Capital Region (BCR), the amount of industrial and commercial waste is estimated, unlike the household waste that is measured. Both in the estimations as in the measurements, the waste flows are strongly aggregated. In order to evaluate environmental impacts from waste collection and treatment and to analyze the valorization potential of current and future waste flows, more data are needed. The data should be available in a framework that links material consumption and waste flows in a consistent way. This work aims to develop a regional material flow model, accounting for the material consumption and waste flows in the BCR. The material flows shall not only be presented as as input/output balance flows entering and leaving the region, but reveal the flows within the economy (production), between the economic activities (final consumption) and households and the interaction with stocks (accumulation). Therefore, an environmentally extended multi-regional input-output model (EE-MRIOM) that shows the BCR in relation to others regions, is suited to analyze flows in such a detail. That model shows material flows in economic and environmental extension in physical units. The current work extends the environmental data on resource uses and emissions with new data on waste flows. These waste flows originate from the regional waste register that provides the amount of collected and treated waste in a high resolution. The data from the waste register was treated in order to improve their reliability and accuracy by operating quality and consistency controls. Then, the cleaned data were converted to be connected to the BCR material flows data and to the current BCR EE-MRIOM. The first results show that previous estimations of waste flows for the BCR were underestimated due to a weak resolution of data. The new data is now linked to economic activities and households, so that future amounts of waste can be estimated based on IO based metabolic analysis. Projections of household consumption expenditure and development in economic sectors are necessary to run such analysis. The developed model supports the estimation of future waste generation, but is also able to analyze the environmental performance of the recycling sector and other waste treatment/valorization options.

LCA of territorial contexts: upscaling the Life Cycle Thinking to business clusters, neighborhoods, urban agglomerations and territorial entities (P)

MO224
Supporting LCA upscaling to neighborhoods by using BIM and GIS tools
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The Life Cycle Assessment (LCA) methodology becomes the main approach used for the environmental assessment in the construction field. The researchers and stakeholders made important efforts over the last years to adapt the general LCA principles to the specificity of the construction works and also to create and share data, especially at product and building scales (e.g. INIES database, HQE Performance). Nevertheless, assessment of the neighborhoods impacts on environment is still a difficult issue in LCA studies. Major challenges are the huge amount of data to be used for an accurate assessment and the need to integrate the specific geographic context, especially for some impact categories, such as the biodiversity. The aim of this work is to show the potential of coupling a LCA Building tool with the BIM (Building Information Modeling) and GIS (Geographic Information System) tools for the environmental assessment of neighborhoods. A proof of concept for calculating environmental impacts of a neighborhood has been developed and used for a test case (100 ha project in Grenoble area, France). The workflow allows us to compare and classify different scenarios for the construction project. Methodological developments were carried out especially in order to i) harmonize the models of different components of the neighborhood and to ii) integrate the impacts on the biodiversity. For the first issue the methods used are based on dynamic energy simulation and LCA Building models. For the second issue, the methods are based on the LCA models for the ex-situ/embodied impacts on the biodiversity and on graph theory for the in-situ impacts due to the habitat fragmentation. DIMOSIM (DIstrict MOdeller and SIMulator) was used to perform energy simulations. ELODIE software (LCA based) was used to support the calculation for the environmental impacts: climate change, water consumption, waste production and embodied impacts on the biodiversity. Q-GIS tool served for the calculations of the habitat fragmentation impacts on the biodiversity. The EVE platform - a BIM and citygml viewer allowed us to support the integrated approach, to harmonize the input data and to represent the overall results. This approach allowed us to accompany and support decision processes through an integrated modeling and informative visualization of the results. Future work will focus on the improvement of methods and interoperability of simulation tools.

MO225
Globally-differentiated land use flow inventories for life cycle impact assessment
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LCA can be a useful methodology to assess land use impacts, e.g. change in soil quality and impacts on biodiversity. Over the past five years, several life cycle impact assessment (LCIA) methods have emerged, with specific focus on including spatial differentiation. Although normalisation is an optional step in LCIA, the increasing emergence of land use indicators and LCIA methods should be accompanied by corresponding normalisation references to enable putting assessment results in perspective. This requires building consistent global inventories of land use flows that can accommodate the level of spatial differentiation of the methods and include both land occupation and transformation flows. To the authors' knowledge, such inventories however are currently missing. Here, we aim to bridge this gap and develop global country-specific inventories of land use flows. Using data from public databases, e.g. FAOSTAT, we developed methodologies for determining occupation and transformation flows for agricultural flows, forestry flows, urban flows and other land use flows such grassland and shrub land. Transformation flows were calculated by determining the differences between the same occupation flows across different years. The resulting inventory, representative for the year 2010, covers 19 occupation flows and 15 transformation flows for 226 countries and territories. With the exception of specific land use types, the coverage of available land occupation data per land class is above 98.8% at global scale. We illustrated the application of this inventory for LCIA purpose by calculating normalisation references for the ILCD recommended method developed by Mila i Canals et al. and by evaluating the most contributing land use classes and countries to the impacts. It highlighted the importance of urban land occupation and transformation, contributing to close to 50% of the impact altogether. For other tested LCIA methods (not addressed here), different distributions were observed, thus demonstrating a strong dependency on the limited number of land use types that typically drive the impacts and on the need to differentiate inventories into specific land use classes. The developed land use flow inventory is thus believed to be the most comprehensive, differentiated and up-to-date inventory available today, and its completeness is deemed sufficient to match ongoing developments in land use LCIA and help make large-scale footprint studies.

MO226
Driving 'Beyond LCA' Metrics for Net Positive Cities
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The authors' present CASE studies using metrics and tools that will allow us to measure progress towards true sustainability across the whole built environment. The scale of applications shown range from products to buildings and infrastructure for cities. The presentation addresses applications of the novel life cycle benefit analysis (LCBA) concept. These include exemplars vital for establishing how developments and products can deliver net positive carbon outcomes. It will show how to integrate planetary boundaries as well as adress calls to correct LCA methods for Climate Change Assessment from 25 Non Government Organisations (NGOs) from 6 countries. The presentation also demonstrates the need for metrics that will allow us to measure progress towards true sustainability across the whole built environment from products to buildings to cities. We now measure with tools like life cycle analysis (LCA) which focus on the negative impacts of systems and products. Such measures ignore realisation that LCA techniques currently do not enable or show us how we may create buildings that deliver the positive ecological system benefits essential for safe operating space within planetary boundaries. Such benefit analysis and metrics are needed to allow measurement of aspects of cities' operation that re-constitute ecological services and generate health benefits. Metrics cover operations and performance including oxygen generation, water cleansing, VOC absorption, pollution reduction, etc. All these are essential in establishing how developments can contribute to delivery of ecological services and healthy environments. The generation of scientifically robust, whole of life metrics, needs to done in a way that does not mean the whole industry must become instant experts in healthy chemicals and life cycle analysis. The authors will present various tools that enable industry-wide, easy comparison of products and with

adaption, assessment of developments and explores what is needed to constitute truly sustainable communities and cities. The aim is to demonstrate reasoning underpinning: the need for a change to the way LCA is conducted; the difference between Life Cycle Impact and Life Cycle Benefit Analysis (LBCA) which LCBA metrics are being proposed how LBCA is being used now for products and projects The need for 'beyond LCA' metrics is demonstrated in practical CASE studies including of building scale LCBA for net carbon positive outcomes

MO227

Life Cycle Assessment of the social housing policy in Mexico from 2000 to 2012

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During the period from 2000 to 2012, Mexico implemented a social housing policy based on the construction of horizontal housing (individual two-story houses), in far places from urban centers, which has generated environmental, social and economic problems in the Mexican metropolitan areas. In order to identify, in an objective and systematic way the environmental impacts associated to the housing policy, in this study a Life Cycle Assessment, of the 7,610,258 million of Social Interest Housing, was conducted. The system analyzed consists of the stages of extraction of raw materials, manufacture of materials of construction, construction, use and end of life of the houses. The study considers the 59 metropolitan areas of Mexico where the housing were constructed; classifying them in small, mediums, big and megalopolis. The life cycle inventory was generated with national data from the Statistical National Institute (INEGI), governmental reports and bibliographic references, and data gap were complimented with Ecoinvent database. The life cycle impact assessment evaluated the impact categories of climate change, acidification, formation of photochemical oxidants, fossil depletion, eutrophication and human toxicity. The ReCiPe method and Sima Pro 8.1 software were used. In the stage of use of the houses, the environmental impacts were calculated according to the use of electricity, fuel consumption according to the travel distance from the housing to the work places and transport mode, LP Gas combustion, wastewater disposal, treatment of fermentable urban solid waste. The results showed that 75% of the environmental impacts are generated during the use of the house. Stages of extraction of raw material, manufacture of construction materials and construction of the social housing contribute with 20% of the impacts and the end of life stage generates 5%. The contribution to the environmental impact of the use phase was distributed as it follows: passenger car transport contributed to human toxicity, freshwater eutrophication, fossil depletion and climate change. Energy consumption contributes to eutrophication of fresh water, human toxicity and climate change. Bus transportation contributes to the fossil depletion, human toxicity, formation of photochemical oxidants. LP gas consumption contributed to fossil depletion, climate change and acidification. According to the results, the use of private transport is the activity with higher impacts.

MO228

Comparative Life Cycle Sustainability Assessment of Urban Water Reuse Alternatives

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With many communities approaching the limits of their readily available water supplies, wastewater reclamation and reuse is an attractive option for extending available water sources. Municipal wastewater is a significant and continually available source of non-potable water and its treatment for reuse purposes has, indeed, become commonplace in many parts of the world. A centralized approach to wastewater reclamation and reuse is the prevalent practice worldwide, but interest in distributed systems is on the rise. Potential gains from transition to a distributed approach may include better water security, lower energy demand, lower maintenance costs and encouragement of local community engagement, among others. The goal of this research is to perform a multi-objective comparative sustainability analysis of alternative scenarios, of different centralization scales, for urban domestic wastewater treatment and reuse, using a life cycle perspective. Four alternative scenarios referring to a hypothetical Israeli city in 2050, are compared. The first scenario represents a current practice of centralized wastewater treatment with no urban reuse, while the other three represent urban domestic water reuse for toilet flushing and urban irrigation, in growing measures of de-centralization of treatment: at city-, cluster- (320 households) and building- scale. Two of the alternatives employ separation at the source of greywater. The developed approach incorporates a combination of three life cycle methodologies (LCA, S-LCA, LCC) assessing the three pillars of sustainability, and a Multi-Criteria Decision Analysis methodology (AHP - Analytical Hierarchy Process). AHP serves as a tool for eliciting and integrating expert judgements in setting the weights for the social indicators, defined within the social assessment and for weighting the three sustainability dimensions and their sub-categories. Results show that urban reuse may reduce environmental impacts and in some cases also the annual costs of the water-wastewater system and increase its social benefits. Specifically, greywater reuse at cluster level was pointed out as the most sustainable alternative among the four compared. AHP proved to be a very effective participatory instrument in overcoming the obstacles of aggregating the results of the multiple criteria of life cycle methodologies to a single score. This may be very valuable for making the outcomes of such assessments accessible to decision makers.

MO229

Promoting Sustainable Circular-Economy Regions through an integrated assessment of Energy, Water and Waste flows (Sant Feliu Llobregat project, SFLL)

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Circular economy is key to achieve sustainable growth in a resource-efficient and environmentally-sound way. The role of water, energy and waste management sectors are central to drive a circular economy – i.e. to collect, treat and return secondary resources and recovered energy back into the cycle of production and consumption. This project aims at implementing Circular Economy concept in a region for emerge opportunities between actors through input and output resources flows analysis (water, energy, materials) to shift linear economy model (take – make – dispose) for circular economy model (close the loops). SFLL is located at 12 km west of Barcelona, counts on 43,000 inhabitants. There are 257 industries of several sectors located in 8 industrial areas. The key actors involved were the City Council, Baix Llobregat Agricultural Park, Collserola Natural Park, water utility and 11 industries located in the industrial areas of El Pla and Les Grasses. After the analysis of the data provided by the key actors and Catalan agencies for waste and energy, up to **10 circular economy opportunities were identified** and discussed with the City Council and local stakeholders. To give an example, they involved reclaimed water for municipal purposes, using biomass from local forest for District Heating or joining industries for a shared waste management system and infrastructure. In just five months of project and with the time and technological limited data collection process, the experience showed the **potential of engaging local key actors in order to perform a holistic analysis of a region under the circular economy model**. However, some barriers were identified involved the **Legal Framework** as understanding applicable legal issues, rights and obligations of public administrations and private companies is essential for implementing a circular model. This project has contributed to "implementing the circular economy concept" developing an innovative methodology to be applied at regional scale for boosting actions to "ensuring the use of secondary resources / scrap materials / wastes in other industries or value chains" and is in line with Roadmap to a Resource Efficient Europe. "Cascading use of materials, reuse, recycling" resources (energy, water and raw resources) and "eco-design, repair and remanufacturing" products or services have been the expected outputs (circular measures or actions) in this project.

MO230

The response relationship of surface water environmental quality to land use in the near field of rivers in Argun River basin of Sino Russian border area

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The response relationship of surface water environmental quality to land use in the near field of rivers is one of the important components of land use environmental effects research. Based on monitoring water quality and land use data, this paper focused on quantitative relationships between land use pattern and water quality in Argun River basin of Sino Russian border area. Firstly with the help of GIS spatial analysis tool, strip types of contributing zones with different buffer radii were developed around the monitoring sites for water quality assessment, and the proportions of four types of land use types were extracted from the buffer zones. Then correlation and redundancy analysis (RDA) methods were used to analyze the relationship between water quality and land use patterns within buffer zones, and a comparison between mountainous areas and urban built-up areas was made. Results showed that the land use structure in the river side area in Argun River basin had great impacts on water quality parameters. Urban construction land showed negative effects on water quality, and was the dominant factor for river pollution in Argun River basin. The forestland exhibited a significant positive relation with water quality, especially with Permanganate index and ammonia nitrogen suggested that the closer forestland tend to improve water quality. The impacts of cultivated land on the river water quality were complicated. In addition, under different terrain conditions in Argun River basin, there were some differences in the way and to the degree of land use influencing the variables of water quality in the same contributing zone scale. RDA demonstrated that there existed obvious scale effect caused by different land use types. The study results should provide practical guidelines for the management of water environment in Argun River basin of Sino Russian border area.

MO231

Environmental and economic life cycle assessment comparison between side-stream treatments for ammonium rich wastewaters

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The Nijhuis Ammonia Recovery system (NAR) is a novel technology capable of

removing ammonia from wastewater and simultaneously producing ammonium sulfate, which can be used as fertilizer. The goal of this research was to quantify the environmental impacts of the NAR system and to compare these with the impacts of a combined SHARON (partly nitrification)-Anammox plant. For this, a Life Cycle Assessment (LCA) was performed with a functional unit of 'the treatment of 1 kg of total dissolved nitrogen inflow (kg $N_{total,in}$)'. First, an LCA of the entire NAR process was performed. Subsequently, the environmental impacts of the conventional manufacturing of ammonium sulfate by the mainstream, so-called Haber-Bosch (H-B), process were subtracted from the environmental impacts of the NAR's ammonium sulfate production in order to obtain only the nutrient removal impacts and fairly compare the outcomes with other WWTPs. A digestate flow capacity of 270m^3/day and 2.5 kg of NH_4^+ concentration per m^3 of digestate and 80% of ammonia removal efficiency was considered. A transport distance of 150 km was included for the transport of ammonium sulfate from the H-B plant to the customer, while for the NAR system it was assumed to be produced on-site. The ReCiPe2008 LCIA method (H) at midpoint level was used to quantify the environmental impacts of the NAR system. Preliminary results show that, when offsetting the H-B avoided impacts, the NAR's impacts on climate change (CC) are >35% lower than the values of the two-step Anammox. Electricity during the use phase and production of chemicals needed for the NAR process contributed most to the CC impacts. The avoided CC impacts by eliminating the need for off-siteammonium sulfate production are more than 75% of the total impacts caused by the NAR system. A scenario analysis revealed that the NAR is less CC environmentally and economically impacting with higher NH_4^+-N concentrations, while the SHARON-Anammox is less CC impacting under lower NH_4^+-N concentrations. When analyzed from a circular economy perspective, the NAR system surpasses the function of only recovering ammonia from ammonium rich streams. The produced fertilizer can be commercialized and used locally representing a surplus and a win-win situation for the NAR's investors. When choosing a side-stream system, trade-offs between N removal performances, costs and environmental impacts should be considered. Therefore, strategic implementation and a case-by-case analysis are recommended.

MO232

Economic and environmental impacts considering global scale supply chain of flood in Thailand 2011

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According to IPCC 5th report, it is expected that the frequency of flood occurrence will be increased. So mitigation and adaptation of climate changes are payed attention in the world. One of the typical adaptation method for flood is creating walls, but it is build them all over the world. Damage, loss and environmental impacts related to flood affect not only occurred country but also all over the world because of globalization. However, detail analysis of damage, loss and environmental impact related to flood are not existed. Generally, disaster of damage and loss estimate only direct affect, but in fact, indirect affect is existed. In this study, we estimated about damage, loss and environmental impact related to flood which focus on 2011's Thailand flood. We applied flood disaster data and Eora database which produced by university of Sydney team. Eora database is Multi-regional Input-Output table and, it cover 190 countries and about 15000 sectors. As results, damage, loss and environmental impacts are obtained, and these are shown below. Damage is 6.6E+07(1000US$), loss is 1.6E+08(1000US$) and CO2 emission is 1.3E+07 (t-CO2). As a rate of totall damge and loss, Thailand is 70% and 60%. Others occured 30% and 40%, but almost rate occurred Asia or Europe. By analizing, these results are expected to know each country/sector risk.

Life Cycle Data and Modeling Developments: challenges and solutions (P)

MO233

Circular economy: Recycling glass fibre reinforced composites (GRP) according to EN 15804 Module D (End-of-Life) through applied LCA scenarios

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The Circular Economy Package of the European Commission comprises of an action plan covering the entire cycle from creation, utilization to waste management and secondary raw materials. Main objectives include targets to reduce landfill, promotion of economic instruments to encourage development of greener products through recovery and recycling schemes. Composite materials are used by a wide range of major industrial sectors (automotive, construction, electronic, renewable energy, etc.). Recycling of plastic-based materials is thus expected to gain momentum, for both thermoplastic composites (recycled by grinding into small particles, followed by injection moulding) and thermoset composites. However, contrary to thermoplastic composites, the thermosetting composite cannot be reshaped again by heating. Thus the challenge of finding the best suited reconversion option, especially with a market estimated at more than 300.000 tons GRP waste per year in Europe. Current solutions include landfilling (readily available but less preferred through legislative changes), incineration (not ideal due to significant ash rests that need to be landfilled and varying energy content), incineration with energy recovery, mechanical processing (as filler in different products), chemical recovery (separation of glass fiber, fillers and resin) or co-processing (mix of material and energy recovery for the production of cement). The study presents research on GRP pipe systems (manufactured with standard and PET-recycled resins) and focuses the analysis of LCIA benefits and impacts through application of Module D, according to EN 15863. Several scenarios are assessed at product level (pipe) and system level (1000m network of pipes, couplings and shafts): reference scenario, recuperation of factory waste through incineration with energy recovery, complete recuperation of factory waste and product end-of-life through energy recovery, system end of life through energy recovery as well as different scenarios for mechanical re-utilization as per currently applied solutions, etc. Scenarios are then compared against each-other and solutions scrutinized through Module D and Circular Economy Package requirements. Final results provide therefore valuable comparative insights to recycling of GRP pipe systems through a variety of available market solutions.

MO234

Activity-based footprinting: putting LCA at the center of company policy.

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The traditional approach of creating separate LCA reports for single products has several drawbacks, e.g.: -a static report gives only a snapshot of the current situation; -an LCA of a single (green) product does not say much about where hotspots lie in a company's overall environmental impact; -data can easily be manipulated: e.g. a company can claim that all of its renewable energy goes into one product, while using fossil energy for all others; -input data often relies on databases with general, non-company specific data; To address these issues, we developed a novel top-down LCA approach, in which data input on energy, materials and emissions is entered at company level, and then allocated to all processes and products. This allows companies to create LCA calculations for their complete portfolio of products within one study. Moreover, this powerful tool gives complete and detailed insights into how resources are used within the company, and which process and product optimizations would yield the highest environmental benefits. This empowers LCA practitioners to actively contribute to company decisions on resource efficiency. The on-line application allows suppliers to share environmental profiles with customers, without having to reveal sensitive data such as exact product compositions. This allows companies to make real-time comparisons between the performance of suppliers, and effectively creates an on-line marketplace for green products. The EcoChain approach is illustrated with several case studies of companies in the construction sector.

MO235

The development of Life-cycle Health Impact Assessment methods for Indoor Air Quality Pollution and Urban Air Pollution

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According to the World Health Organization report, the number of deaths due to indoor air quality pollution and urban air pollution estimated, as 6.5 million people until 2012. In LCIA research, although several studies have tried to develop methods for outdoor and indoor air pollution, these impacts have not been compared nor aggregated among them. In this research, we focused on a development of LCIA method for indoor air quality contamination. DALY, disability adjusted life year was chosen as an indicator expressing human health damage. Damage factors developed were applied to case studies to verity the reliability and availability of our results.

MO236

Review schemes and reviewers' selection criteria for the Life Cycle Data Network

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A review framework and coherent review schemes are fundamental for increasing validity and comparability of reviews, lower the efforts and costs for review, and support higher acceptance of LCA. The International Reference Life Cycle Data System (ILCD) Handbook provides detailed guidance on consistent and quality-assured LCA and LCI, as well as rules for reviewer's eligibility criteria. A simplified set of entry level (EL) requirements for LCI data to be hosted in the Life Cycle Data Network (LCDN) was released in 2012, and the Environmental Footprint (PEF/OEF) scheme is adding further compliance rules. The aim of this paper is to define the eligibility rules for reviewers, in the different schemes. At global scale, in 2011 the in the framework of the UNEP/SETAC life cycle initiative, was released the "Global Guidance Principles (GGP) for Life Cycle Assessment Databases" The document provides also details on review that were futher expanded within the UNEP umbrella. The Reviewer Registry, which is part of the Resource Directory of the EPLCA http://eplca.jrc.ec.europa.eu/ResourceDirectory/ is hosting info about reviewers and reviewer teams, and is able to assess the eligibility 3 different schemes (ILCD, ILCD EL, and PEF/OEF), the tool can be expanded also for the GGP Scheme. The minimum requirements are calculated on the following parameters: years of experience in LCA methodology, practice, verification and audit; sectorial expertise (NACE main sector). Additional practice on LC based approaches, papers, methodological and data development, PhD or Master thesis related to LCI/LCA practice. \n Different schemes have different minimum score to be reached, in order to be eligible as independent or team member reviewer. For e.g. ILCD EL the minimum required experience to be an independent reviewer are: 2 years of experience in verification/audit, 2 reviews performed, 2 years of experience in LCA methodology and practice, participation in 4 LCI works, at least 2 years of sectorial experience (public or private organisation, for each sector of eligibility) a single reviewer is eligible as member of a team, if is fulfilling at least 1 of the mentioned requirements. Different schemes are also defining the rules on the number and type of reviewers required (e.g. independent internal, independent external, or dependent internal reviewer). Those eligibility criteria are case-sensitive and therefore not implemented in the Reviewer Registry.

MO237
Roadmap toward addressing and communicating uncertainty in LCA
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Life Cycle Assessment (LCA) models for quantifying emissions and resources used as part of the life cycle inventory (LCI) step and for characterizing related impacts on human health, ecosystem quality, and natural resources as part of the life cycle impact assessment (LCIA) step together contribute considerable uncertainty and variability at different assessment phases. These contributions have led to questions about the ability of LCA results to be used in decision-making. Mainly, variability is related to spatiotemporal, technological, and interspecies and inter-individual differences, while uncertainty is further related to input data, model selection and choices, amongst other aspects. Currently, methods exist to assess and assign uncertainty and variability on LCI data as well as LCIA characterization results. However, often uncertainty is only assessed and reported qualitatively, is not comparable across impact categories and not consistently assessed and reported across levels of detail. Furthermore, many existing methods and models do not report uncertainty at all or limit their uncertainty assessment to a sensitivity analysis of selected input parameters, while ignoring variability, model uncertainty, and uncertainty related to choices and human errors. As part of the LCA Capability Roadmap, a committee of nearly 40 contributors under the auspices of the SETAC North America LCA Interest Group is currently working to identify research needs in the area of ill-characterized uncertainty. The group has investigated current best LCA practices, such as refinements to the pedigree matrix used to assess LCI data quality. In parallel, in the frame of UNEP-SETAC Life Cycle Initiative flagship project on providing Harmonization and Global Guidance for Environmental Life Cycle Impact Assessment Indicators, a task force focusing on uncertainty aspects has been established. This task force currently investigates best practices in existing LCIA methods and works on a minimum set of criteria for consistently reporting uncertainty in LCIA. These best practices and state of the art will be presented along with proposed milestones toward providing guidance of how to address and report uncertainty in LCA to improve current practice. Feedback is encouraged.

MO238
Glucose production: influence of the datasets and of the long term emissions on LCA results
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The aim of this study is to have a good understanding of the environmental impact of glucose production. Glucose is generally produced from corn or wheat. Since agricultural processes are known to be difficult to evaluate by LCA, the results obtained with two different LCA databases, Gabi and EcoInvent, are compared in this work. The production of glucose from raw materials can be divided in two steps: the agricultural step allowing the cereal production, and the conversion step including the extraction of the starch from the plant and its hydrolysis into glucose. Preliminary results underline the high impact of the agricultural step, so a special attention has been paid to these data. Specific Belgian data collected by the Walloon Agricultural Research Centre (CRA-W) (2014) have been used as primary data (yield, amount of fertilizers, etc.), either using EcoInvent or Gabi datasets background data to model fertilizers, diesel consumption, etc. A third model was built using only data available in Ecoinvent for corn and wheat cultures. For the conversion steps, literature data have been used along with some industrial data. Based on these multiple sources, it is possible to compare the LCA results for the production of 1 kg of glucose for three different cases. The results underlined that the differences between the two databases are smaller than the differences between specific data (Belgium data) or non-specific data (Ecoinvent) for the agricultural steps. Nevertheless, in some impact categories, the differences between the two databases remain high. The presentation will underline where these differences are coming from. This leads to also analyze the differences between background data such as energy generation or fertilizer production. Moreover, special attention has been put on the influence of long-term emissions, in the Ecoinvent database. As these emissions have a large influence in some impact categories, we have to clarify if we should include them or not in view of comparison with GaBi database. Moreover, the Ecoinvent model and the GaBi models have been realized in two different software (Simapro and GaBi, respectively), therefore, some checks have been performed to see if some differences can be induced by the software. In conclusion, this presentation will underline which is the sensibility of the results to parameters not controlled by the LCA practitioner, such as the datasets hypotheses, the software differences, etc.

MO239
Exploring prospective scenarios of water supply mix
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Recent research has been conducted for the development of a water supply mix (WSmix) for LCA. The WSmix is a mix of water resources and related technologies to meet a user at a specific time and location at a worldwide scale. The WSmix was inspired by 1) the concept of the electricity production mix in LCA 2) specific literature on inclusion of water in the LCI and the Quebec tap water supply mix, integrated in ecoinvent v3.2. The proposed WSmix represents a snapshot of what is embedded today in a m3 of water in terms of resources consumed and pollutants emitted to produce it. However, changes in water resources, mainly driven by changes of climate and socio-economic factors, will occur in the future. Climate change affects directly water availability, vegetation needs and land-use, while socio-economic factors like projected population growth directly impact future water consumption patterns, and water demand by different users. In this context, the WSmix should be able to consider those changes over time, in particular for products or infrastructure with long lifespans. Thereby, it would provide temporally projected water mix profiles, capable of reflecting the water-use environmental impacts under projected future conditions at any given location in LCA.The global model for future scenarios of WSmix builds on the current WSmix and presents the following specificities: i) per country (and watershed) ii) seasonal, annual, ii) for domestic, extended to agriculture and industrial uses, iv) for conventional and non-conventional water resources, v) for conventional and emerging new technologies. Several data sources have been analysed (water management plans, climate models, etc). Based on that, three major global variables seem to be the main driving forces for the modelling of future WSmix: climate change, growth and migration of populations and economic development. Literature review and data analysis on the future evolution of water resources and water needs has been done for several countries at a watershed level. Preliminary results on future scenarios for WSmix for several countries have been constructed. Aiming to cover more watersheds and countries, a global model for the forecast of WSmix scenarios has been created. The prospective WSmix model developed is an essential element for water-use impact assessments in LCAs for long lifespan products or infrastructures. Also, it can have a relevant applicability on water resource planning and management.

MO240
Best Proxy: A New Methodology for Selection of LCI Dataset to Achieve Regionalized LCA
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The common practice of using generic or country-specific LCI datasets for LCAs for which site-specific LCI datasets are not available can result in inaccuracies and affect the relevance of the LCA results. On the other hand comprehensive site-specific LCI datasets require considerable time and effort, and data that are rarely available at the level of desired detail. We present a new best proxy methodology for systematic selection of the most appropriate LCI dataset for a specific site, out of the available LCI datasets for a specific background process. The aim of the methodology is to select the dataset that will result in the LCA impact scores that are closest to "true values", at only a small fraction of the effort needed to generate a comprehensive site-specific LCI dataset. When used as a background process for an LCA of a product/service at that site, the selected dataset will evidently lead to better estimations of LCA results than a generic or random country specific LCI dataset. The selection process is based on the concepts of characteristics associated with each dataset for the background process and of "distance" between LCI datasets in the characteristics space, where the missing LCI dataset of the analyzed site is also represented by a set of descriptive characteristics. A rigorous mathematical approach is used to define the "distance" between any two datasets for a specific process in the characteristics space. The dataset with the minimum distance to the site with the missing dataset is the selected as the best proxy dataset. The methodology is general and can be applied to various background processes. The methodology is demonstrated and validated on a model of water supply systems that serves as a case study using a harmonized set of 23 published LCA studies and corroborated on a harmonized set of electric power stations fired by coal. The results demonstrate the validity and the predictive power of the methodology. The methodology has an incorporated learning capability, demonstrated with the case of the Israeli water system LCA. The cost-effectiveness of the methodology is demonstrated by comparing the effort needed to carry out a site-specific LCA of the Israeli water supply system to the effort of implementing the model for one site. The model developed for water supply systems can be used in sites for which site-specific LCAs are not available. The methodology can be

used to develop similar models for other background processes.

Fate, risk assessment and management of natural toxins: state-of-the-art, challenges and future prospectives (P)

MO241

Ecotoxicological risk assessment of microbial biopesticides under EU Regulation (EC) No. 1107/2009

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The biopesticide industry is expected to reach more than 7% of the total crop protection market (> $4.5 billion) by 2023, and projected to grow most rapidly in Europe and Latin America. In fact, most new active substances under current evaluation in Europe are biopesticides. The approval and authorisation of microbial biopesticide active substances and products in Europe falls under the same regulation as conventional chemical pesticides, EU Regulation (EC) 1107/2009. Guidance, including that for the environmental risk assessment, is available for microbials. Nevertheless, the data requirements and additional guidance for microbials are often difficult to interpret or limited to vague criteria of assessment. Furthermore, there is a scarcity of EU test guidelines for the conduct of ecotoxicological studies testing microbials. These challenges are coupled with the relatively low regulatory experience and/or biological understanding of biopesticides across the Member State regulatory authorities and the European Food Safety Authority (EFSA), often leading to delayed decisions and requests for further data that may be inappropriate for certain substances. In the meantime, whilst new guidance is in development, applicants must continue with only the limited guidance to place their products on the EU market. Here, the ecotoxicology sections of recent EFSA conclusions for microbial biopesticide active substances were reviewed. Our review aimed to identify common areas of concern and trends in the type of data gaps identified during the peer review of the ecotoxicological risk assessment. Prior to any new submission it is recommended that a detailed data gap analysis is conducted, specific to the substance. However, these initial findings may assist future applicants to better focus their submissions to avoid some of the common difficulties and pitfalls. Furthermore, these observations may help those involved in the development of new guidance to understand where applicants and regulators would benefit from better defined data requirements and assessment criteria.

MO242

Quantification of the carcinogen ptaquiloside in complex matrices

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Ptaquiloside (PTA) is found in several ferns, most notable in the Bracken ferns (*Pteridium* sp.). PTA cause urinary bladder cancer in bovines and is suspected to take part in the formation of Human gastric cancer. In Brackens, PTA is found in roots, rhizomes, fronds and spores (up to 5% w/w). PTA is a water soluble norsesquiterpene glycoside, and can cause contamination of soils and drinking water resources. PTA can enter the Human food chain via contaminated milk, meat, blood and directly through Bracken based traditional food products. PTA is analysed using HPLC-UVVIS, GC-MS, LC-MS or LC-MS/MS. Analysis is based on *direct* quantification of PTA in purified extracts or *indirectly* after converting PTA into pterosin B or bromo-pterosin. PTA is rather stable under pure analytical conditions and can be quantified by MS-detectors. However, using UV-detectors or GC-based techniques for analysis of PTA in complex matrices requires conversion of PTA into pterosins to obtain acceptable LOD, separation, and GC-suitability. Pterosins are easier to extract from complex matrices. PTA is converted to pterosin B by acid hydrolysis after aglycon formation under alkaline conditions. Quantification often assumes a 1:1 ratio between PTA and the pterosin. However, this is not always the case. Artefacts like chloro-pterosin may form. The purpose of this project was study the conversion rates for a range of commonly used acids to hence making it possible to compare studies using indirect quantification of PTA. PTA (500ppb) was deglycosidated (1mL PTA soln. was added 75μL 1M NaOH, heated for 1hr (35^{o}C)), cooled to room temperature and added 75μL acid (triplicate; 1.0M/2.5M/5.0M): HCl; H_2SO_4, HNO_3, TFA, FA, HBr, and HAc. PTA and pterosin B was quantified by LC-MS. Deglycosidation caused formation of pterosin B and 2 intermediates. The highest conversion ratio was obtained using 1.0M acids. FA, HAc and TFA performed best (35-45% higher conversion compared to traditional HCl conversion) indicating severe underestimation of the true content of PTA due to formation of chloro-pterosin. Using HBr results only in formation of 40% of the TFA method. Acid reaction with Br is used for quantification of PTA in meat and milk and for GC-MS analysis. The poor conversion of PTA into bromo-pterosin indicates possible severe artefacts in such methods. Studies using indirect techniques should therefore be treated with caution and older studies based on 1:1-conversion ratios should be reassessed.

MO243

Determination of lipophilic and hydrophilic marine biotoxins in seawater with analytical methodologies based in HPLC-HRMS

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Marine biotoxins are secondary metabolites produced by different phytoplankton species. These compounds are generated in hazardous amounts when harmful algal blooms (HABs) take place. About 75 different species have the capacity to produce biotoxins, that can bioaccumulate along the food chain, causing a variety of gastrointestinal and neurological illnesses to consumers. With the aim of determining the occurrence of lipophilic and hydrophilic marine biotoxins in the seawater, two analytical methodologies have been developed and presented in this work. The selected lipophilic biotoxins were Okadaic acid (OA) and related dinophysistoxin-1 (DTX1), pectenotoxin-2 (PTX-2), azaspiroacids-1, 2, 3, 4 and 5 (AZA-1,2,3,4,5), and yessotoxin (YTX) and related homoyessotoxin (hYTX); while hydrophilic toxins were tetrodotoxin (TTX), domoic acid (DA), gonyautoxins-2,3 and 5 (GTX-2,3,5) and related decarbamoylgonyautoxins-2,3 (dcGTX2,3), decarbamoylsaxitoxin (dcSTX), and neosaxitoxin (Neo). The analytical method consisted, first, in the filtration of the seawater sample. The particulate and the aqueous phase were extracted independently: the former by ultrasonic assisted solid-liquid extraction (UASE) and the latter by solid phase extraction (SPE), employing OASIS-HLB cartridges for the lipophilic biotoxins and activated charcoal for the hydrophilic ones. Because of the very different chemical nature of the selected compounds, the chromatographic separation required the use of two columns: a reverse-phase LC column was employed for lipophilic toxins and a HILIC column for the hydrophilic toxins. In both cases, the chromatographic system was coupled with a heated electrospray ionisation source to a high resolution mass spectrometer with a hybrid quadrupole-Obitrap analyser (Q-Exactive, Thermo Scientific). The presented methodology was successfully applied to real samples from the Ebro Delta Basin (North-East of Iberian Peninsula) and the preliminary environmental results will be discussed.

MO244

Uncertainty sources for a reliable quantification of Alexandrium species by molecular techniques

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Alexandrium is a cosmopolite genus of marine dinoflagellates that includes the largest number of toxic species, i.e. able to synthesize toxic compounds. The most common toxic compounds produced by *Alexandrium* toxic species are related to Paralytic Shellfish Poisoning (PSP), which are targeted by shellfish monitoring programs worldwide; other toxic compounds produced by some species of *Alexandrium* are considered emerging risks. Risk management related to PSP toxins, as well as other marine toxins, is based on the identification and quantification of the source organism. Utermöhl method is currently used in routine and is the reference method; nevertheless, it has some important constraints such as the time spent per sample (24h minimum of pre-treatment, i.e. sedimentation of the cells, plus 2-3h of average per sample analysis), the resolution (optical microscopy is not suitable for the species identification of all microalgae), the small amount of sample (maximum 100 mL), and the subjectivity (the taxonomic expertise of the analyst is clue). Alternative methods are assessed in order to reduce the time and expertise and increase the resolution and the number of samples and data obtained. Many of these methods are based on molecular techniques; regardless of the end point (Sanger sequencing, qPCR, Next-Generation Sequencing), they have important common steps. Molecular techniques have proven to be suitable for the identification of microalgae species, but their quantification is still difficult. *Alexandrium* species have been used to test the DNA extraction and quantification steps, and compare the results with cell concentration in the original sample, in order to identify critical issues to be addressed if molecular techniques are aimed at quantification of toxic microalgae. Possible solutions or strategies will be suggested for further research.

Higher tier approaches in the risk assessment of plant protection products and their links to protection goals (P)

MO245

Enclosure set up: an old system as new semi-field approach for common voles to assess their risk on plant protection products

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For plant protection product (PPPs) registration the risk of birds and mammals needs to be assessed. For all crop groups at different plant growth stages the small herbivorous mammalian scenario – the common vole – needs to be covered according to the respective Guidance for Birds and Mammals (EFSA 2009). Due to the species ecology like 100% herbivorous diet, small home ranges and light body weight, the risk calculation often fail to demonstrate a save use of PPPs and for the same reason classical refinement options like portion of time or diet in treated area, recommended by EFSA (2009) as higher tier refinements do not differ in their values to be applied in vole assessments compared to the first tier assessment. Therefore, in the majority of cases, the risk can further be addressed in weight of evidence approaches only. Here enclosures are a potential option to monitor even individual fate in the long-term of common voles exposed to PPPs in a treatment versus control set up in natural habitat. The enclosure set up enable to control e.g.

the time point of study, the vegetation cover, or the densities of vole at study start. Hence, the outcome is a comparison of control and treatment groups on individual based data. This approach is able to close the gaps of large scale field effect studies on common voles.

MO246

Collection and analysis of pesticide residue data for pollen and nectar to be used for bees' risk assessment

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In 2013 the "Guidance document on the risk assessment of plant protection products on bees" was issued by the European Food Safety Authority (EFSA). Due to the limited availability and high variability of residue data at the time of GD development, worst-case assumptions were made which resulted in a conservative exposure assessment. Recently, as novel and more standardized studies had been conducted, where the residues in nectar and/or pollen were measured, EFSA funded a project to analyse these newer data. The outcome of this project will be used to address the identified data gaps and to update the risk assessment methodology for pollinators for enhancement of the first tier assessment reliability. A detailed screening of the data submitted and evaluated for the peer-review process under Reg. (EC) 1107/2009 at European level was performed in order to identify the new relevant studies (conducted from 2010 onwards) for pesticides residue data on pollen, nectar and other bee's products. During the screening procedure 314 pesticide active substances have been screened (AIR II, AIR III and New Active Substances) and 125 relevant studies have been identified in the respective regulatory documents (DARs, RARs, Registration Reports, *etc*). Following the prioritization procedure by using predefined selection criteria, 45 of these studies have been selected and their data have been captured in an MS Excel database specifically developed for the needs of this project. The selected studies have been conducted in different countries inside and outside Europe (France, Italy, Spain, UK, USA, *etc*) for different crops (*P.tanacetifolia*, grapes, rapeseed, orchards, *etc*) while the analysed matrices for pesticide residues were mainly nectar, pollen (collected from bees, hives or directly from the flowers), larvae, wax, honey, flowers and plant parts. Furthermore, RUD values have been calculated from the measured residues values in each matrix and the respective application rates. For studies and matrices where the residue dissipation was followed by sampling in a sufficient number of time points after pesticide application, DT_{50} and DT_{90} values for each matrix have been calculated. Finally, potential correlations between residue levels in pollen and nectar and physicochemical and environmental fate & behaviour properties of the active substances have been examined.

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MO247

Can red seeds become green? Refining exposure assessment to minimize risks of pesticide-treated seeds to farmland birds

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Seed coating with pesticides is a widespread practice that reduces the necessity of spraying and the amount of active substances released to the environment. However, this practice poses a risk to granivorous birds, which could become intoxicated because of the ingestion of seeds that are not properly buried during sowing. After experimentally studying the toxicity of coated seeds to red-legged partridges (*Alectoris rufa*), we present a new project, REGRESEEDS, to characterise the exposure of farmland birds to coated seeds, and to validate measures focused on mitigating risks through the reduction of the exposure. We will start validating non-invasive biomarkers to estimate pesticide exposure in order to use them as a monitoring tool in the field. Exposure assessment will be conducted, on the one hand, through GPS-tracking of partridges to study their spatial ecology and to determine how the occurrence of coated seeds in the field influences habitat use by these birds. On the other hand, we will analyse partridges' diet by metabarcoding of excreta in order to relate diet composition to pesticide levels in faeces. Once exposure scenarios are determined, we will evaluate the efficacy of different measures, like burial of coated seeds or supplement of clean food, to reduce the risk of poisoning of partridges and other farmland birds. In order to make these measures applicable to the entire EU, efficacy of proposed mitigation measures will be tested in Spain, France and the UK. The project aims at resolving a problem that affects different sectors of the society, which has motivated the involvement of farmers, hunters, pesticide manufacturers, governmental agencies, nature conservationist groups and environmnetal management business. Its timeliness is fundamented in the upcoming review of the EFSA Guidance Document of Risk Assessment of Birds and Mammals. Financed by the Spanish Ministry of Economy and Competitiveness

MO248

Data collection for the estimation of ecological data and residues on food items to be used in pesticide risk assessment for birds and mammals

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In order to enable a consistent and harmonised implementation of the requirements of Regulation 1107/2009 and its related regulations concerning the placing of plant protection products (PPPs) on the market, Guidance Documents (GD) are developed by the EU Commission and the European Food Safety Authority (EFSA), with help of EU Member states experts. The current EFSA Guidance Document for the risk assessment of birds and mammals (2009) follows a tiered approach. For the first tier assessment a range of scenarios have been developed. The scenarios are combinations of ecological characteristics of 'generic focal species' and other factors relevant to exposure, e.g. the type and structure of crop, and the type of formulation of the pesticide product. The GD further gives a range of options for higher tier risk assessment in case a low risk cannot be identified in the course of the first tier assessment. The refinement steps comprise the identification of real focal species (FS), the use of measured residues and residue decline in food items, field information on the composition of diet (PD factor) and field information on the proportion of an animal's daily diet obtained in a real habitat treated with pesticide (PT factor). Since the GD came into effect, a considerable amount of new data has been produced, which can potentially add valuable information to the birds and mammals risk assessment. In 2016, a project was therefore begun in order to compile ecological (FS, PT, PD) and residue data, so that it may be appropriately and consistently used in the future. A large amount of data has since been collected from the public literature and from sources summarising studies submitted to EFSA and/or member states for product or substance registration purposes. Available studies are screened and categorised according to their relevance and reliability. Finally, the data are organised and summarised in unified databases. Once completed, the databases can be used by the authorities, and will also serve as a basis for updating and reviewing the EFSA GD on risk assessment for birds and mammals. A summary of the work is presented on the poster.

MO249

Protection of field margin strips established as ecological focus areas in Germany

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Ecological focus areas which are established as field margin or buffer strips are one possibility to fulfill the greening requirements within the common agricultural practice in the EU. The general value of such structures for biodiversity and ecosystem services is well known. Additionally, synergies do exist with risk mitigation requirements in the context of the regulation of pesticides. Such strips can be used to protect e.g. water bodies or terrestrial non-target habitats from pesticide entries and also have a positive effect on recovery potential. This has been widely acknowledged during the discussions on risk mitigation options in the MagPie workshops. However, to ensure that these strips can fulfil the intended ecological function, a minimum standard in the protection from entries of agrochemicals such as pesticides would be required. However, the regulation does only cover the direct application of pesticides in ecological focus areas but not drift or run-off entries from the treated field into such areas. As there are no clear rules for a minimum protection standard of strips as ecological focus areas, farmers are uncertain whether they have to protect such strips from pesticide entries. This might be one reason why German farmers up to now rarely use field margin strips as ecological focus area. The poster will clarify the legal requirements concerning ecological focus areas (field margins strips). Furthermore, we will propose an approach how field margin strips can be implemented and simultaneously be protected by a combination of drift reducing techniques and spatial requirements.

MO250

MAgPIE toolbox, practical examples of its application in aquatic risk assessment

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The risk assessment for aquatic organisms in the EU is based on a comparison of the Regulatory Acceptable Concentrations (RAC) and the Predicted Environmental

Concentrations (PEC) in surface waters. The former are based on the ecotoxicological effect endpoints and appropriate assessment factors. The latter are estimated accounting for different possible entry routes, through spray drift at the time of application or through drainage and/or runoff following application. In case PECs are higher than the RAC, risk mitigations can be implemented to reduce the final concentration of the PPP in surface waters and prevent any adverse effects on aquatic organisms. Many different risk mitigations can in principle be adopted during applications to reduce spray drift (e.g. buffer zones, drift reducing nozzles) or to reduce a future runoff from a treated area (e.g. vegetated buffer strips). However, the still limited communication on the effectiveness of these risk mitigations, makes that the number of tools actually implemented at national level highly variable among member States (MS) and flexibility to use new or alternative risk mitigation options is rare. A 2-steps MAgPIE workshop was organized in April and November 2013, under the auspices of SETAC and the European Commission, in order to develop a toolbox of risk mitigation measures designed for the use of pesticides for agricultural purposes, and thus contribute to a better harmonization of their development and use within Europe. This presentation will show a practical example of how the MAgPIE toolbox could be used to mitigate FOCUS R scenarios in the aquatic risk assessment. Based on FOCUS Step 4 calculations, the contribution of spray drift and runoff to the PEC_{SW} for R scenarios was determined, as well as the percent reduction of these components of the PEC_{SW} necessary to achieve a final PEC lower than the RAC. Using the point system in the MAgPIE toolbox, the required mitigations were established. MAgPIE offers the possibility of harmonising the risk mitigation options available in different MS by promoting a standardised toolbox for adoption at EU level. The MAgPIE toolbox offers a simple approach for the identification of suitable risk mitigations that can be implemented in different MS. The toolbox offers flexibility in the selection of adequate risk mitigations so that different options or combination of mitigations can be used while maintaining the required protection level for aquatic organisms.

MO251

Geraniol: case study of a substance approved under REACH, the Plant Protection Products, Biocides and Cosmetics Regulations

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Geraniol is a terpenoid compound present in many essential oils. It is used as fragrance ingredient in cosmetics for its rose-like odour. In addition, it has insect-repellent and insecticidal properties, which make it an effective active substance for use in plant protection products and biocides (product types 18 and 19). Geraniol is registered under REACH (Reg. (EC) No 1272/2008), the Cosmetic Products Regulation (Reg. (EC) No 1223/2009), the Plant Protection Products Regulation (Reg. (EC) No 1107/2009) and the Biocidal Product Regulation (Reg. (EU) No 528/2012). These regulations are governed by different regulatory bodies and competent authorities (e.g. DG GROWTH vs. DG SANTE, EFSA vs. ECHA), resulting in different regulatory requirements and assessment strategies. We present a case study of the data requirements and potential for data waiving under the different legislations, a comparison of the timelines for review/renewal of active substance dossier and we highlight differences in approaches for exposure and risk assessments. The aim is to elucidate where consistency between the different legislations is lacking and where there is scope for further harmonization.

MO252

The use of recovery endpoints in aquatic risk assessments: what about isolated waterbodies?

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Under Regulation (EC) 1107/2009, it is increasingly difficult to demonstrate acceptable risks to aquatic organisms at Tier I. Therefore, it is often necessary to turn to higher-tier approaches, with one such tool being mesocosm studies. Under the current aquatic guidance document (EFSA 2013), two types of endpoint can be generated from mesocosm studies: no observed effect concentration (NOECs) and no observed adverse effect concentration (NOEAC). The NOEAC represents a concentration at which aquatic communities will experience adverse effects but can recover within a period of time. The acceptability of different magnitudes of effects depends on the time period required for recovery i.e. a small effect can last for months, a medium effect can last for weeks, a large effect can last for days. Regulatory authorities are often cautious about using NOEAC endpoints in the risk assessment due to concerns that recovery in real waterbodies may not occur, particularly in physically isolated waterbodies that do not benefit from unaffected areas upstream acting as a source for recolonization. In situations where the impacted surface water body is isolated from other water bodies, the time required for an impacted community to recover may be higher, which could impact whether an effect is considered acceptable under EFSA (2013). Individual species traits outside of their sensitivity to pesticides such as dispersal ability, timing of dispersal and generation time play an important role in their ability to recovery; however, these factors will also determine whether a species is present in isolated water bodies prior to a disturbance event. The aim of this poster is to examine whether the concerns of regulatory bodies that isolated waterbodies may not recover following disturbance are valid. This will include investigating the extent of waterbody isolation in different agricultural landscapes using GIS techniques. We will also examine the effect of isolation on biodiversity and species identity. This poster will also discuss the processes by which species establish within isolated waterbodies e.g. terrestrial adults, wind, translocation *via* other species (birds, mammals), periodic flooding events. We hope that these investigations will contribute useful information which can help regulators to make more informed decisions on recovery endpoints.

MO253

Practical Experience of MDD Analyses for Mesocosm Studies: Challenges in Deriving Reliable Endpoints from Tricky Taxa

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In 2013 the European Food Safety Authority (EFSA) introduced the use of the Minimum Detectable Difference (MDD) as part of the statistical analyses of mesocosm studies to increase transparency and reliability of endpoints, thus aiding regulatory decisions for plant protection products (PPPs). Following this, Brock *et al.* (2015) published an interpretation of these guidelines, providing more detailed elements for the evaluation of experimental ecosystems. CEA have reanalysed a number of species abundance data sets from mesocosm studies using these methods to comply with the still novel regulations. Here we highlight our practical experiences from these analyses. In particular, we provide examples of challenges in the application of MDDs to evaluate micro and mesocosm results and explore solutions to these where guidance is still in development. The derivation of endpoints with the help of MDDs can be straight forward when analysing highly abundant taxa with a clear monotonous dose response. However, for taxa with low or variable abundance, interpretation of effects can be tricky and often subjective. While for a given species there may be sufficiently low MDDs to meet the criteria for Category 1 or 2 (effects can be reliably determined), effects may not be properly demonstrable due to the inconsistency of the data. This uncertainty may result in a decrease in original study endpoints, especially where observed effects are isolated and recovery cannot be determined. In these instances, the biological relevance and abundance levels must be considered, and both a conservative yet realistic approach is required. Furthermore, guidance suggests significant increases should be assessed irrespective of the MDDs, potentially introducing uncertainty from an array of poor quality data. We discuss whether or not it is appropriate to include such data. Difficulties also arise where study design does not fit current standards and pre-application samplings may be missing, replication is low or samplings are infrequent in the initial phase of PPP exposure. Although MDDs are a measure of the reliability of statistical significance or lack of significance, they cannot be used solely to derive study endpoints. These always have to be determined by using the raw data, statistics and MDDs in conjunction. Here we demonstrate that this is particularly important when addressing ‘tricky taxa’ to reduce the subjectivity that may affect the endpoints of PPPs.

MO254

Effects of the fungicide fludioxonil on zooplankton in outdoor sediment-spiked microcosms

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A sediment-spiked microcosm experiment was conducted with the fungicide fludioxonil to evaluate the sediment effect assessment procedure proposed by the European Food Safety Authority (EFSA). Endpoints concerned compound fate and responses of sediment microbes, sediment nematodes, benthic macro-invertebrates, zoo- and phytoplankton. Intended fludioxonil concentrations of the spiked sediment were control - solvent control – 60 – 180 – 540 – 1620 – 4860 – 14580 µg/g OC in dry sediment (n=4). Since fludioxonil has a relatively high log K_{OW} (4.1), this substance was expected to bind to sediment, but due to equilibrium processes exposure was expected in the overlying water as well. This poster focusses on fludioxonil concentrations in the water compartment of the microcosms and on responses of zooplankton (Cladocera, Copepoda and Rotifera). Exposure concentrations of fludioxonil in the overlying water gradually increased, resulting in a long-term exposure regime. Four weeks after the construction of the microcosms fludioxonil concentrations in overlying water on average were: 0 (real control) – 0 (solvent control) – 0.76 – 2.33 – 8.46 – 21.06 – 51.79 – 92.52 µg/L. In total 69 different zooplankton taxa were observed in the microcosms. With a NOEC of 180 µg/g OC in sediment, corresponding to 2.33 µg/L in overlying water, the cladoceran *Diaphanosoma brachyurum* showed the most sensitive treatment-related decrease. Other zooplankters that showed treatment-related decreases were rotifers, and the cladoceran *Daphnia longispina*. Cyclopoid Cyclopoda showed the lowest NOEC for a treatment-related increase (540 µg/g OC in sediment, corresponding to 8.46 µg/L measured in overlying water). Other taxa that temporarily increased in numbers due to indirect effects were calanoid Cyclopoda, the rotifer *Synchaeta* sp. and a few cladocerans. These data indicate that in sediment-spiked microcosms chronic exposure to fludioxonil in overlying water resulted in a NOEC for the most sensitive zooplankton population (approximately 2.33 µg fludioxonil/L) that was somewhat lower than the 21-d chronic NOEC reported for *D. magna* (5.0 µg fludioxonil/L). Note, however, that the chronic exposure regime in the water compartment of our study is caused by introducing spiked sediment in the test system, while in edge-of-field surface waters a pulsed exposure regime is expected due to normal agricultural use of this fungicide.

MO255

The Use of Probabilistic Assessment to Determine Risk of Aquatic Plants Exposed to a Herbicide

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Exposure and effects are the key elements in risk evaluations. Deterministic risk analysis uses point values to evaluate risk of non-target biological organisms exposed to environmental stressors and does not take into consideration variations and uncertainties in species sensitivity nor in the environmental conditions that can strongly influence exposure. Accounting for this variability and uncertainty can provide a more realistic view of how stressors impact populations. Probabilistic Risk Assessment (PRA) incorporates uncertainty and variability into the risk assessment paradigm by providing estimates of the likelihood of risk rather than a single point estimate and thus provides a distribution of risk across populations and/or environmental exposure conditions. A case study was undertaken to demonstrate the usefulness of PRA in an aquatic environmental risk assessment. Aquatic plants were exposed to a single herbicide and the distribution of twenty different ErC50 values for the various species were measured and utilized in the assessment. Probabilistic analysis was performed by fitting the distribution of effects and randomly selecting endpoints within that distribution over 10,000 simulations. Similarly, distributions of estimated environmental concentrations were sampled accounting for variable wind speed and application equipment parameters (nozzles and boom height). These Monte Carlo simulations provided percentiles of risk quotients which were then compared to the appropriate risk criteria. Overall, these results demonstrate the usefulness in using probabilistic analysis for higher tier risk assessments to gain an understanding of how variability and uncertainty can influence risk.

MO256

Using Laboratory Microcosms to Address Mesocosm Data Gaps

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Outdoor mesocosms are an excellent resource for producing aquatic community endpoints for Plant Protection Products (PPP). However, in some cases additional information is needed on species that are either not present in the study or identified as particularly sensitive. Higher-tier laboratory microcosm studies can be used as an intermediate between OECD lower-tier ecotoxicology and mesocosm studies to address data gaps and therefore reduce uncertainty for regulatory endpoints if needed. Here we will present two novel laboratory microcosm studies that were conducted at CEA to address data gaps from mesocosm studies and to provide further resolution on a single species endpoint. In our first example, an indoor microcosm was set up to provide additional endpoints for non-standard species. Plecoptera and Megaloptera, which were suggested to be sensitive in the literature, were tested alongside organisms present in the mesocosm to determine comparative sensitivity. This provided a direct link between the higher-tier study and the mesocosm and thus was used to validate the test system. The microcosms were set up and tailored to the specific organisms' needs to replicate their natural environment in order to reduce stress. Outdoor mesocosms allow for free movement of flying organisms. This can potentially result in uncertainty with respect to the causes of population decreases of sensitive species. In our second example, in order to help identify what caused the decrease in the population (toxicity or movement), the effect of a PPP on a mobile species was investigated in a closed system laboratory microcosm. The use of higher-tier laboratory microcosms are effective at addressing mesocosm population data gaps. These studies can be tailored to the individual requirements of the organisms and chemical to produce more realistic exposure scenarios whilst reducing environmental stress.

MO257

Statistical power in higher tier studies. How much is enough?

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Experimental designs with low statistical power are more likely to produce a type II statistical error, whereby existent differences between treatments go undetected. Therefore, the European Food Safety Authority (*1*) requests that a statistical power analysis is performed before higher-tier experiments are carried out. (This requirement also applies for experiments not based on current standard test protocols.) This contribution performs statistical power analyses for several different test systems commonly used for regulatory risk assessments. These test systems are different with respect to the type of statistical analysis (e.g., Student t-test, repeated measures ANOVA, dose-response curves) as well as to the temporal and spatial scale of their experimental designs. This work will also estimate the minimum detectable difference (MDD) between control and dosed treatments (*2*), recently advocated as a post-hoc procedure informing about the statistical power of an experiment. The main objectives of these exercises are (1) to check whether previous experimental designs were sufficiently robust assuming a certain effect size or if their future designs need to be adapted and (2) to inform the regulatory community about inherent variance in test systems and how it can be minimized. Finally, this poster will offer a critical assessment of the feasibility of high statistical power, the regulatory requirements, and the inherent variability of higher-tier ecotoxicological studies within the regulatory framework. (1) EFSA. European Food Safety Authority. *Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology*. EFSA supporting publication 2015:EN-924. 62 pp. 2015. (2) Brock, T.; Hammers-Wirtz, M.; Hommen, U.; Preuss, T.; Ratte, H. T.; Roessink, I.; Strauss, T.; Van den Brink, P. *Environmental Science and Pollution Research*. 2015, *22* (2), 1160-1174.

MO258

SETAC Ecological Risk Assessment Interest Group

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Linking Oceans and Human Health: a new trans-disciplinary research challenge (P)

MO259

Blue Health: linking Europe's aquatic environments with human health and wellbeing

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Over 50% of the EU population lives within 50 km of coastline, while the average urban European lives 2.5 km from a freshwater source (river/lake/canal); this translates into at least 5.4 million jobs and EU GVA of > €500 Billion/year associated just with EU coasts, not to mention those associated with the inland waterways. Although Europe's blue infrastructure is sometimes considered a sub-set of its 'green' infrastructure, this ignores its unique cross-sectoral roles (e.g. river and marine transport, trade, fisheries and aquaculture, tourism, and health). Moreover, growing evidence shows that the health promotion and disease prevention opportunities of the blue infrastructure are distinct from, and in some cases synergistic with, those provided by our 'green' and 'grey' infrastructure. Blue infrastructure may also be more vulnerable to short-term climate and other environmental change and stressors (e.g. rivers drying up, sea levels rising, pollution), as well as becoming of increasing global importance with regards to water availability and quality. What is far less well understood is whether and how urban blue spaces can also play a role in tackling major public health challenges of the 21st Century such as obesity, physical inactivity and mental health disorders. The aim of the Horizon 2020 funded BlueHealth Project is to systematically explore these possibilities, and to investigate whether careful design and implementation of urban blue infrastructures can promote benefits to public health and prevent disease by, for instance, encouraging people to take more exercise, or by helping to reduce the stress and anxiety known to be created by living in highly urbanised settings. Initial evidence suggests these benefits may be substantial and widespread; and may be especially important for vulnerable populations such as children, those with underlying poor health, and those in deprived communities. As yet, however, there has been no attempt to systematically characterise or quantify these benefits, or to use this information to inform and improve the design of urban blue infrastructure to deliver not only the more well-established cross-sectoral goals such as transport and water provision, but also to aid in the promotion of health and prevent disease in the 21st Century. **Acknowledgements** Horizon 2020 funding

MO260

Harmful Algal Blooms, Climate Change and Human Health

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Harmful Algal Bloom (HAB) species produce potent natural toxins which are detrimental to both animal and human health. People are exposed to the toxins through consuming contaminated seafood, as well as through contact with contaminated water and aerosols. Impacts from these biotoxins include gastrointestinal, respiratory and possibly neurodegenerative diseases (both acute and chronic), and in severe cases, death. HABs are increasing in aquatic ecosystems worldwide which may be due to natural and anthropogenic climate change, and changing non-climatic environmental factors (e.g. nutrient loading). These changes could affect future algal bloom frequency, composition and spatio-temporal distribution and, in the UK, may lead to the permanent establishment of historically uncommon HAB species. This could pose an increasing threat to human health from multiple routes of exposure, including the consumption of biotoxin-contaminated seafood. It is therefore important to understand the relationship between HABs, climate and other environmental changes, and human health to be able to project future changes in HAB events to prevent and mitigate health and socio-economic impacts. A scoping review of the topic has been undertaken to map the existing literature and identify knowledge and research gaps. In addition, we will use outputs from a hydrodynamic, biogeochemical coupled model for the northwest Atlantic shelf (NWS AMM7), observational *in situ* and satellite oceanographic data, and UK HAB species monitoring datasets to investigate the links between observed and modelled environmental variability and specific HAB-species occurrences. In the future, UK hospital episode statistics linked with the HAB monitoring data will be used to explore the potential associations between HABs and human health impacts. Finally, climate projections for the UK (UKCP09 based on medium emissions scenarios) will be used to identify projected changes to key environmental variables directly linked to algal bloom development, and thus to HABs and human health risks. **Acknowledgments** Funding provided by the UK Natural Environment Research Council (NERC), the UK Medical Research Council (MRC), and the UK National Institute of Health Research (NIHR)

The role of ecotoxicology in fisheries science and aquaculture (P)

MO261

Halogenated and organophosphorus flame retardants in aquaculture samples

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Flame retardants (FRs) are compounds applied to materials to increase their fire resistance. Polybromodiphenyl ethers (PBDEs) are the most used FRs and can be found in plastics, furniture and electronic devices. They are classified according to their level of bromination. Since PBDEs are not bonded into plastics, but blended, they can leach out and are found in different environmental compartments. PBDEs are persistent and toxic, bioaccumulate and are ubiquitous through long-range transport. They are considered persistent organic pollutants (POPs) by the Stockholm Convention and their production has been banned in Europe and North America. Hence, new brominated FRs (BFRs) are used as substitutes, as well as chlorinated FRs, including halogenated norbornenes (or dechloranes). Organophosphorus FRs (OPFRs) have been applied for decades not only as FRs, but also as plasticisers. The use of OPFRs was almost as high as that of PBDEs and might have increased after the ban of the latter. Additionally, they are just blended into materials like PBDEs. However, limited information about OPFRs has been published. The present study investigates the occurrence of halogenated FRs (HFRs) and OPFRs in water (n = 27), sediments (n = 24) and mussels (n = 17) from aquaculture farms in Greece, Italy, Norway, Portugal, Spain and the United Kingdom. Samples were collected between spring and summer 2016. The analytical methods monitored 8 PBDEs, 3 emerging BFRs (DBDPE, HBB and PBEB), 5 dechloranes (*syn*- and *anti*-Dechlorane Plus and Decs 602, 603 and 604) and 8 OPFRs. For the sample preparation freeze-dried samples ̜except for water—and internal standards were used. HFR extracts were analysed by GC-MS/MS. Method recoveries for HFRs ranged from 51-88 % and LODs and LOQs were 0.0003-0.96 ng/g dry weight (dw) and 0.001-4.78 ng/g dw, respectively. OPFR extracts were analysed by GC-MS and LC-MS/MS. Method recoveries for OPFRs ranged from 47-112 % and LODs and LOQs were 0.02-1.25 ng/g dw and 0.05-3.44 ng/g dw, respectively. All groups of compounds were detected in 75-100 % of samples for each matrix, except for emerging FRs in mussels, which occurred in less than 53 % of them. The banned DecaBDE is still the main PBDE found in sediments, followed by its substitute DBDPE. Dechloranes were in a similar range to DBDPE in all matrices. OPFRs showed a distribution similar to waste-water derived contaminants, with higher values near effluent discharge points.

Toxicology and ecotoxicology: bridging the gaps (P)

MO262

Biotransformation of clofibric acid in zebrafish embryos (Danio rerio) as determined by liquid chromatography-high resolution-mass spectrometry

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The zebrafish embryo (ZFE) is increasingly used in ecotoxicological and toxicological research but detailed knowledge of its biotransformation potential is still limited and thus the comparison to other better known species limited. This study focuses on the biotransformation capability of ZFE at different life-stages using the pharmaceutical compound clofibric acid as study compound. Liquid chromatography with quadrupole-time-of-flight mass spectrometry (LC-QToF-MS) was used to detect and to identify the transformation products (TPs). In screening experiments, a total of 18 TPs were detected and structure proposals were elaborated for 17 TPs, formed by phase I and phase II metabolism. Biotransformation of clofibric acid by the ZFE involves conjugation with sulfate or glucuronic acid, and, reported here for the first time, with carnitine, taurine, and aminomethanesulfonic acid. Further yet unknown cyclization products were identified using non-target screening that may represent a new detoxification pathway. Sulfate containing TPs occurred already after 3h of exposure (7hpf), and from 48h of exposure (52hpf) onwards, all TPs were detected. The detection of these TPs indicates not only the fast uptake of the parent substance clofibric acid but also the unexpected high activity of phase I and phase II enzymes already at these very early life-stages and partly before the actual development of a functional liver. The results of this study outline the high biotransformation potential of the ZFE with respect to the analysed xenobiotic. Biotransformation of test chemicals in toxicity testing with ZFE may therefore need further consideration. Similarities but also differences to other test systems were observed and will be discussed and classified in terms of a possible alternative to species used in toxicological studies.

MO263

Experiences with the Draft EFSA (2016) Guidance on Benchmark Dose Calculations: Reliability of BMD for bird and mammal reproductive risk assessment

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In 2009 EFSA published an opinion on the use of benchmark doses (BMD) as a more robust and reliable alternative to the NOEL. EFSA has now released a new draft guidance on benchmark doses (EFSA, 2016), in which a number of issues with regard to the previous recommendations have been solved by proposing a new methodology for dose-response model selection and endpoint calculation, which previously resulted in unrealistic endpoints. Furthermore, some dose-response models have been eliminated. Based on a fungicide case study, we assess how the changes of the new guidance document affects the realism of BMD caluclations. We show that in some cases biological expert decision may still be needed to obtain realistic endpoints.

Alternative approaches to animal testing for (eco)toxicity, and the regulatory application of the 3Rs in chemical risk assessments (P)

TU001

An intestinal fish cell barrier model to assess absorption of poorly soluble organic chemicals in vitro

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Fish, like other aquatic organisms, accumulate organic chemicals present in water (bioconcentration) and via the food chain (biomagnification). The intestine of fish is considered a major route of exposure and uptake of chemicals, especially for poorly water soluble chemicals. To establish a versatile *in vitro*model, the fish intestinal epithelial cell line, RTgutGC, was seeded on permeable supports to study permeation of poorly soluble chemicals, such as some fragrance compounds. From an experimental point of view, working with fragrances poses certain difficulties due to their hydrophobicity (logK_{OW} > 3.5) and their volatility (log HLC > -5), resulting in potential losses in conventional experimental exposure setups either by adsorption to exposure vessels or by evaporation. We here report on the development of a novel exposure chamber for permeation experiments, designed to allow well-defined testing of hydrophobic and/or volatile chemicals, using the fragrance Damascone beta as example. The permeation of Damascone beta was measured in two different exposure setups: at 19°C with or without a chemical sink (= PDMS coated stir bar) in the receiving compartment. *In vivo* a chemical sink is represented by blood proteins and flow, which likely maintains a concentration gradient as driving force of permeation. Mass balance analysis revealed a full recovery of Damascone beta in cell-free conditions but substantial losses in the presence of cells. Experiments at 4°C indicated the involvement of energy dependent processes, such as biotransformation, as source of loss. Permeation data showed that the RTgutGC cell layer presents a barrier for Damascone beta permeation and that adding a chemical sink to the receiving chamber increases transferred mass. Calculated permeability coefficients were in a similar range to permeabilities of a fluorescent model compound with similar size. In summary, the *in vitro* barrier model of the fish gut established with RTgutGC was successfully used to measure the permeation of the fragrance Damascone beta. A specific exposure chamber was constructed which offers a well tunable system with predictable exposure concentrations, which is especially important for reliably testing hydrophobic and volatile substances. In a next step, the calculated permeability coefficients shall be included in toxicokinetic models as input parameter to improve bioaccumulation assessment.

TU002

Effects of repeated exposures to fine particles (PM0.18-2.5) and ultrafine particles (PM0.18) on inflammatory chronic lung diseases exacerbation

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Many epidemiological studies have highlighted an association between levels of ambient particulate matter (PM) and hospital admissions or even mortality related with exacerbation of asthma and chronic obstructive pulmonary disease (COPD).While the role of inhaled PM in exacerbating these pathologies has been clearly established, pathophysiological mechanisms initiating and maintaining airway inflammation are not well understood. Reported health issues seem to be mostly caused by the finest particles, due to their ability to diffuse deeper in the respiratory tract, where pulmonary clearance is less effective. There have been numerous experimental studies demonstrating the toxicity of fine particles ($PM_{2.5}$) through oxidative stress-induced airway inflammation, epigenetics modifications or cell cycle alteration, however, a few of studies have paid close attention to the ultrafine fraction ($PM_{0.1}$), which can represent more than 70% of total number of particles. Particles also attain new properties at nanometric scale. Because of their high specific surface area, $PM_{0.1}$ are likely to be more biologically reactive and, therefore, to be involved in large part in the toxic effects of PM. In the present study, we conducted *in vitro* assays, exposing differentiated models of bronchial epithelial cells (HBEC), sourced from healthy (n=4), asthmatic (n=4) and COPD-diseased (n=4) donors, to one or repeated low doses of $PM_{2.5}$ and $PM_{0.18\text{-}2.5}$. Culture media were collected to assess cytotoxicity and secretion of inflammatory mediators, mRNA were extract to analyze transcriptome expression profile. Chemical characterization of PM have identified some reactive compounds such as trace metal elements, and organic compounds, which are likely to generate oxydative stress at the cellular level to lung tissues. Characterized and differenciated models of epithelial bronchial cells were use to analyse secreted inflammatory mediators. The analysis revealed that diseased bronchial cells overreact compare to normal bronchial cells at low dose of exposure, particularly COPD cells. $PM_{0.18}$ treatment trigger a more accentuate response than $PM_{2.5}$, suggesting that ultrafine fraction of particles air pollution is largely involved in toxic effects of PM. Microarray analysis have showed immune gene expression modulation in each group. Results of this study should contribute to a better understanding of molecular mechanisms governing the respiratory inflammation induced by these pollutants.

TU003

New alternative carcinogenic prediction assay using flatworm stem cell proliferation patterns

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Accurate and reliable carcinogenicity assays are imperative, as cancer risks are directly associated with the type and potency of a compound. A challenge for the development of alternative test methods is the prediction of non-genotoxic carcinogens, which entail different assessments of human cancer risk. The variety of non-genotoxic cancer pathways complicates the search for sensitive and reliable parameters expressing their carcinogenicity. The presented assay enables a simple, rapid and inexpensive prediction and discrimination of both genotoxic and non-genotoxic carcinogens by means of *Schmidtea mediterranea*'s stem cell dynamics. Our methodology entails an exposure to carcinogenic compounds during the animal's regeneration process, and the most striking differences between non-genotoxic and genotoxic carcinogen-induced proliferative responses were detected during the initial stages of the regeneration process, i.e. at the moment stem cells proliferate. We present a two-step-approach that combines *in vivo* adult stem cell proliferation patterns and phenotypic appearances. Based on the observed differences in stem cell dynamics we were able to discriminate between genotoxic and non-genotoxic carcinogens in a selected group of compounds (MMS, 4NQO, CsA, S-PB, MPH, CPZ). More specifically, genotoxic carcinogens were characterized by significantly fewer mitotic cells after 3 days exposure in comparison with a 1-day exposure set-up, while, on the contrary, non-genotoxic carcinogens were characterized by significantly more mitotic cells after 3 days exposure in comparison with a 1-day exposure set-up. The ability to discriminate between genotoxic and non-genotoxic compounds makes this approach unique and with significant added value to current research and drug development.

TU004

Development of an alternative testing strategy for the fish early life-stage test using the AOP network "thyroperoxidase and deiodinase inhibition leading to impaired swim bladder inflation"

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The prediction of reproductive and early developmental toxicity has largely relied on the use of animals. Currently, the fish early-life stage (FELS) test (OECD TG 210) is one of the primary tests used to estimate chronic toxicity of chemicals in fish to support risk assessment. However, there is widespread agreement regarding the need to develop alternative testing strategies. We assessed the feasibility of using an AOP-based approach for developing such an alternative, mechanistically informative testing strategy by investigating whether the AOP framework forms a basis for (1) the development of new non-animal test methods, (2) the prioritization of assay development and (3) obtaining biological context for mechanistic information. We developed an AOP network, with two main pathways leading to effects on the swim bladder of zebrafish and fathead minnow. The swim bladder consists of a posterior and an anterior chamber, which inflate during a Fish Embryo Acute Toxicity (FET, OECD TG 236, early development) and FELS (late development) timeframe, respectively. We defined underlying key events leading to the adverse outcomes and postulated that embryonic thyroperoxidase (TPO) activity is not essential to posterior inflation, while deiodinase (DIO) activity is needed to activate maternal T4 into T3. However, both enzymes are needed at later developmental stages, and inhibition of either enzyme results in impaired anterior inflation. In order to validate this proposed mechanism, we optimized *in vitro* tools for the assessment of the *in vitro* TPO/DIO inhibitory potential of 51 relevant contaminants. Predictions regarding the *in vivo* impact on swim bladder inflation were made, which were validated using 168 hours post fertilization FET and 32 days post fertilization FELS experiments. Results show that only DIO inhibitors decrease posterior chamber surface area at low concentrations and completely inhibit posterior inflation at higher concentrations. The posterior surface area appears to be more sensitive compared to the binary observation of posterior inflation. Finally, FELS exposures with two TPO inhibitors resulted in impaired anterior inflation. Our results are in line with, and increase confidence in, our AOP network. We can conclude that we successfully used the AOP framework for (1) the selection and prioritization of key events and predictive assays, (2) accurate prediction of acute and chronic toxicity and (3) the development of non-animal test methods.

TU005

In Vitro screening to assess biotransformation rates and bioaccumulation potential of volatile and hydrophobic fragrance ingredients

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Bioconcentration is an important parameter in chemical hazard and risk assessment. The gold standard test for determining the bioconcentration factor (BCF) of chemicals in fish is the OECD Test Guideline 305. As this *in vivo* test is of ethical concern due to high animal usage, efforts for non-animal approaches are underway. A key process influencing the BCF is the biotransformation of chemicals, which takes place primarily in the liver of fish. It has been suggested to use subcellular fractions of fish liver such as S9 (supernatant 9 000 x g) or isolated fish hepatocytes for determining intrinsic metabolic clearance rates of chemicals, and to predict from the *in vitro* biotransformation rate values the *in vivo* biotransformation rates k_{met} in the intact fish. Previous research has shown the suitability of S9 fractions and hepatocytes from rainbow trout (*Oncorhynchus mykiss)* to determine intrinsic clearance rates of chemicals. Fragrance ingredients, due to their physicochemical properties impose challenges in particular for *in vitro* test set-up. Therefore, the aim of this study is to further refine those *in vitro* assays using fragrance ingredients and propose adaptations to existing protocols. Problems due to the volatility of test compounds were overcome by modification of the original test protocol. Passive dosing was used to administer highly lipophilic fragrance ingredients to the S9 incubation media without introducing cosolvent. Test substances included notably Muscenone® delta, Tonalide, and Cetalox® which all have log k_{ow} above 5, some of which having already an experimental BCF and were used as further proof of concept. The *in vitro* intrinsic clearance metabolic rate values ranged from 0.5 to 1.2 (ml/h/mg S9 protein), leading to a corresponding refined BCF values ranged from 70 to 300 for Muscenone delta® and Tonalide. This demonstrated that none of those fragrances are likely to bioaccumulate in fish which is confirmed by their experimental BCF. The use of *in vitro* information to derive a BCF can be a good prediction of the experimental *in vivo* BCF, drastically limiting the use of laboratory animals.

TU006

Toxic effects of pesticides used in vineyard on cell lines as well as embryos and larvae of rainbow trout (Oncorhynchus mykiss)

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Many pesticides used in viticulture are entering the aquatic environment *via* runoff and leaching waters. In order to determine the hazard potential of pesticides the early life stage (ELS) test and two *in vitro* tests with RTL-W1 cells were conducted. Cytotoxic effects (MTT assay) and genotoxic effects (modified Comet Assay with Formamidopyrimidine DNA glycosylase (FpG)) on RTL-W1 were investigated with a screening of concentrations (including environmental concentrations) of pure compounds (glyphosate 0.1 and 1 mg/L; copper sulfate 2 and 20 μg/L) and commercial formulations (RoundUp® (RUp) and Bouillie Bordelaise (BB)). Moreover, a mixture of compound was tested on cell lines to simulate a multi-contamination in the environment. In addition, embryonic and larval stages of trout were exposed at the eyed stage until the entire yolk sac absorption to 0.1 and 1 mg/L RUp. In this experiment, various endpoints were measured (e.g. locomotion, genotoxicity and acute toxicity). For the *in vitro* test, no acute toxicity were observed and no significant differences were measured between pure compounds and the respective commercial formulations. However, DNA damage has been demonstrated for the lowest concentration of glyphosate/RUp (0.1 mg/L) and copper sulfate/BB (2 μg/L) in RTL-W1. Similar results has been found in previously investigation using *Anguilla anguilla* exposed to 0.116 mg/L. Specifically for copper sulfate also other studies demonstrated the induction of ROS formation and genotoxic effects in RTG-W1 and zebrafish liver cell line, respectively. The investigation of the mixture of all compounds indicates a potential synergistic toxic effect. Indeed, the first effect was observed with concentrations 10 to 100 times lower than the concentrations required to cause cytotoxicity with the isolated compounds. For the exposure of RUp to embryos and larvae of trout no acute toxicity were observed. Previous studies showed a LC_{50} between 8.3 and 50 mg/L of glyphosate on ELS of trout which is above the concentrations used in our study. However, an increased swimming velocity was detected for individuals exposed to 0.1 mg/L glyphosate when compared to the controls. This effect was previously described as avoidance behavior (stimulation). These results showed the importance to take into consideration toxic effects of mixture compounds which can be more toxic than isolated compounds, and to include the changes in behavior to investigate the hazard potential of chemicals.

TU007

Abiotic Activation of Pro-Electrophiles to Assess their Skin Sensitization Potential in Chemico

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Allergic contact dermatitis (ACD) is a widespread environmental and occupational health disease, caused by chemical substances that induce sensitization through dermal contact. Typically, ACD is ini-ti-ated by electrophilic compounds that are able to penetrate the *stratum corneum* to form antigens by reac-tion with nucleophilic moieties of skin proteins. Therefore, the electrophilic reactivity of a sub-stance often correlates with its sensitizing ability. This correlation is practically applied in chemoassays such as the direct peptide reactivity assay, which can be used to discriminate between sensitizers and non-sensitizers.[1] Besides electrophiles, another group of potentially sensitizing agents is given by pro-electrophiles that can be transformed to sensitizing electrophiles either through autoxidation or me-ta-bolic oxidation. Assessing the skin sensitization potential of such compounds with non-animal methods is still a challenge, because many currently used assays do not include a respective instrument for ac-ti-vation. Our presentation introduces an abiotic activation system that is based on a synthetic catalyst, and demonstrates its application in combination with a chemoassay[2] for a predictive assessment of the skin sensitization potential of organic pro-electrophiles, thus com-ple-menting existing integrated testing strategies.[3] [1] Gerberick GF, Vassallo JD, Bailey RE, Chaney JG, Morrall SW, Lepoittevin J-P 2004 Development of a Peptide Reactivity Assay for Screening Contact Allergens. *Toxicol. Sci.* 81: 331-343. [2] Böhme A, Thaens D,Paschke A, Schüürmann G2009. Kinetic Glutathione Chemoassay to Quan-tify Thiol Reactivity of Organic Electrophiles – Application to Unsaturated Ketones, Acrylates, and Propiolates. *Chem. Res. Toxicol.* 22: 742-750. [3] Rorije E, Aldenberg T, Buist H, Kroese D, Schüürmann G 2013. The OSIRIS weight of evidence approach: ITS for skin sensitisation. *Regul. Toxicol. Pharmacol.* 67: 146-156. *Acknowledgement* – The authors thank BMBF-funded project ProHapTox (FKZ 031A422A and 031A422B) for financial support.

TU008

Evaluation of toxic and neurotoxic effects of sediments from 6 coastal systems in Mexico

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In our country, the studies of the presence and effects of contaminants in aquatic systems are scarce, even though their degree of contamination has increased due to the contribution of pollutants produced by industrial, agricultural and oil activities, and by the development human settlements and tourism activities. The objective of this study was to detect the presence of compounds with toxic and neurotoxic effects in the sediments of 4 coastal lagoons located in the Gulf of Mexico (La Mancha, Farallón Lagoon, El Llano and Verde Lagoon, Veracruz) and 2 beaches Located in the Mexican Pacific (Papanoa and Las Gatas, Guerrero). For the assessment of sediment toxicity, bioassays were carried out with nauplii of *Artemia franciscana* and neonates of *Daphnia magna* and for the detection of neurotoxic compounds an *in vitro* assay was used with sediment extracts and homogenates of foot tissue of *Pteria sterna* bivalve, in this test was evaluated the Inhibition of activity AchE enzyme. In the lagoons located in the Gulf of Mexico, the degree of toxicity was: El Llano> La Mancha> Verde lagoon> Farallón lagoon. On the beaches was: Las gatas > Papanoa. The percentage inhibition of AchE activity varied from 55 to 14% in the tests with the sediments. The sediments that had the greatest neurotoxic effect were those collected on the Las Gatas beach and in the La Mancha lagoon. The results obtained in toxicity and neurotoxicity bioassays agree with the pollutant levels recorded at sampling sites. Therefore the evaluation of toxicity and neurotoxicity is a useful tool for biomonitoring studies.

TU009

Electrochemical activation of pro-electrophilic skin sensitizers in a chemoassay approach

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Skin sensitization is an immunologic process that can result from the covalent binding of an organic electrophile to a skin protein. Therefore, exposure to electrophilic compounds may induce a respective allergic contact dermatitis (ACD) as type-IV allergy. At present, the murine local lymph node assay (LLNA) is the method of choice for evaluating the skin sensitization potential of chemical compounds. Approaches for replacing this animal test include integrated testing strategies, employing in silico and in chemico methods. The latter are based on the fact that for certain model nucleophiles such as glutathione, their reactivity toward electrophilic compounds can be taken as surrogate for the electrophile-protein reaction leading to skin sensitization. Whereas this approach may work fine for intrinsically reactive sensitizers, it would overlook so-called pro-electrophiles that gain their electrophilic reactivity only after uptake in the skin through metabolic activation. In this communication we present a method to mimic the oxidative activation of potential skin sensitizers to reactive metabolites using electrochemistry. The discussion includes experimentally determined reactivities of respective derivatives and their comparison with LLNA data from the literature. The authors thank the BMBF-funded project ProHapTox (FKZ 031A422A and 031A422B) for financial support.

TU010

An alternative approach to Fish Embryo Toxicity (FET) test using the three-spined stickleback

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The Fish Embryo Toxicity test using zebrafish (*Danio rerio*) (zFET) is a recently adopted OECD test guideline (TG236) that utilises embryo mortality as an endpoint. Published data has demonstrated a good correlation of LC50s compared to the widely used fish acute toxicity test (TG203). The zFET records more measurable data than embryo mortality (e.g. deformities), but in its current form it does not include measurement of transcriptional changes stemming from embryonic chemical exposure. This additional endpoint is fully compatible with the zFET test and importantly can expand its current application as a toxicity test to include assessment of chemicals with a specific mode of action such as endocrine disrupting chemicals (EDCs). The three-spined stickleback (*Gasterosteus aculeatus*) is a useful sentinel species in chemical risk assessment, particularly for EDCs, presenting endpoints for both oestrogenic (vitellogenin) and (anti)androgenic (spiggin) chemicals. Spiggin is a glycoprotein produced by the kidney in breeding male sticklebacks and is under androgenic control. The aim of our studies was to expand the scope of the FET test by including gene expression changes as well as developing the stickleback FET (sFET). Here, we report sFET responses to various chemicals as well as global gene expression (Agilent microarray) following exposure to selected EDCs. Furthermore, we studied gene expression changes during developmental stages up until the point of free-feeding larvae. The results highlighted the suitability of the sFET as a screen test for EDCs. In addition, the sFET presents additional advantages compared to the zFET: A full control of fertilisation via *in vitro* fertilisation (vs. natural spawning) The presence of a strong genetic sex marker in this fish species The option of conducting the sFET in full marine, brackish and freshwater tests A tolerance to a wide range of low temperatures equivalent to those in natural environments in this fish species \n

TU011

CON4EI: Consortium for in vitro Eye Irritation testing strategy

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Measurement of **ocular irritancy** is a necessary step in the safety evaluation of industrial and consumer products. Assessment of the acute eye irritation potential is therefore part of the international regulatory requirements for testing of chemicals. Until now, no single alternative test was capable of identifying the different ocular effects observed in the *in vivo* Draize eye test, as a consequence distinguishing between the different UN GHS categories remains challenging. The objective of the **CON4EI project** is to identify strategic combinations of alternative test methods within a tiered-testing strategy in order to replace the *in vivo* Draize eye test. Therefore, a set of **80 reference chemicals** covering the most important *in vivo* drivers of classification, balanced according to the physical form (38 liquids and 42 solids) and representing different chemical classes, was tested in eight *in vitro* test methods. The set was composed of 15 chemicals not requiring classification (No Cat) and 65 chemicals requiring classification (27 Cat 2 and 38 Cat 1). The performance with regard to the *in vivo* drivers of classification of the following methods was evaluated individually: **BCOP** and **BCOP-LLBO**, **ICE**, **EpiOcular EIT**, **EpiOcular ET-50**, **HCE EIT**, **STE** and **SMI** test method. In a second step, two by **two agreement between test methods** was evaluated to identify similarities between methods. Finally, different test methods were **combined into a testing strategy** and the **performance** was evaluated. This analyses provided evidence that **different testing strategies** are possible. For example, a combination of the **BCOP and HCE EIT** test method resulted in an **accuracy of 81%** with a **Cat 1 sensitivity of 86.3%**, **Cat 2 sensitivity of 63%** and **100% specificity**. Furthermore, none of the Cat 1 chemicals were identified as No Cat. This was **an improvement over the stand alone assays** that cannot differentiate between the different categories. Similar results were obtained when the BCOP was combined with HCE EIT or EpiOcular EIT. This research is funded by CEFIC-LRI. We acknowledge Cosmetics Europe for their contribution in chemical selection.
Keywords: CON4EI, STE, eye irritation/corrosion, ocular irritation assay, *in vitro* novel irritation testing; testing strategy \n

TU012

Prediction of phenol ecotoxicity using an appropriate classification into Mechanisms of toxic action.

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The group of phenols is often seen as the representative family exerting the mode of action of polar narcosis, while it actually includes several mechanisms of action. When trying to model the acute toxicity of phenols to fish, daphnids and algae using only one hydrophobicity descriptor (i.e. log Kow or water solubility), this group cannot be predicted by a single hydrophobicity-based QSAR model accounting for the polar narcotics. More precisely, a significantnumber of phenols are more toxic than this model would predict. In order to be able to predict it accurately, several steps should be followed: first the phenols have to be correctly separated according to their real mechanism of action in the different aquatic species. Secondly, the compound toxicity can be predicted using the corresponding local QSAR model. This QSAR model should be developed with high quality data and with compounds acting through the same mechanism of action. As a consequence, accurate predictions can be given, thus replacing animal testing with good confidence. We have defined the following categories for phenols: membrane destabilizers class 2 (polar narcotics), oxidative phosphorylation uncouplers, RedOx-cycling compounds, proreactants (compounds that will be rapidly metabolised into uncouplers in this case), but also endocrine disrupting agents and cyclooxygenase inhibitors. Oxidative phosphorylation uncouplers can be clearly separated from other compounds based on their pKa. The toxicity of this group of compounds could be modelled accurately with only water solubility as a descriptor. On the other hand, 1,2- or 1,4-dihydroxybenzenes are RedOx-cycling compounds, which are more toxic. As part of DAMIER, a French funded project, potential polar narcotic compounds were studied including alkyl and alkoxy-phenols. These compounds showed consistent relationship between their aquatic toxicity and hydrophobicity. Moreover, since the primary and secondary amines are classified as membrane destabilizers class 2 (polar narcotics) they can be gathered with those phenols that follow this same MechoA. From a toxicity perspective, this work shows chemicals must be compared with their respective MechoA and not only according to their structural similarity.

TU013

Predictive QSAR models to assess the toxicity of pirperidine derivatives to Aedes aegypti mosquitoes

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Aedes aegypti mosquitoes can transmit many diseases such as dengue, yellow fever, chikungunya, and recently Zika, which are responsible for millions of deaths every year. Intensive use of insecticides for mosquito control led to unwanted environmental side effects such as contamination of ecosystems, effects on non-target organisms and increasing mosquito resistance. This results in a critical concern for finding new insecticides of high activity, but devoid of these problems. In this poster we illustrate the development of quantitative structure-activity relationships (QSARs) for the prediction of acute toxicity to female adults *Aedes aegypti*, of 33 piperidinic compounds (derived from the generic structure cyclo ($C_5H_{10}N$)-CO-Carbon chain). Models were developed in compliance with the OECD principles for the Validation, for Regulatory Purpose, of (Q)SARs. Activity data were taken from a recent study (Pridgeon et al. 2007) and converted in pLD_{50} (μM/mosquito). Multiple approaches were compared i.e. MLR-OLS, Support Vector Machine (with either linear and radial kernel), Projection Pursuit Regression and K-Nearest Neighbours. Best results were obtained with a 4 parameter model, particularly involving Barysz matrix and BCUT descriptors. A second model, using Kier et Hall atom-type E-State indices, although slightly less performant, was more readily interpretable as to the influence of present structural elements (alkyl or aromatic substitution on the Nitrogen cycle, presence of a vinyl group, length of the carbon chain). The proposed models had good fitting and robustness (i.e. R^2 of about 0.80 and Q^2loo of about 0.70). Their predictive ability was established by external validation with random splitting of the data into five independent pairs 80/20% (training/prediction set). In spite of important conceptual differences (i.e. modelling performed in the original descriptor space or using projections in either larger or lower dimensionality space), the various methods led to convergent results. The best performances were observed with MLR-OLS and SVM. Results from this study provide relevant information on the structural moieties that enhance/inhibit toxicity of piperidinic compounds to mosquitoes. This information and the proposed models are particularly relevant to orient the design and predict the activity of new candidate molecules specific for *Aedes aegypti*. Moreover they can be useful to support hazard assessment of similar compounds.

TU014

Reducing animal products in metabolisation studies - S9 subcellular fractions produced from cells in chemically-defined medium

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Metabolisation is an important process in organisms to detoxify xenobiotics. However, this process can also lead to the production of toxic metabolites. Therefore, for toxicity studies as well as environmental safety assessment of chemicals metabolisation has to be taken into account. Various *in vitro* test systems simulate the liver metabolism with liver S9 subcellular fractions. Most commonly, the source of S9 subcellular fractions for *in vitro* testing are rats. To obtain a highly active metabolic liver fraction the animals are fed with xenobiotics like *ß*-naphthoflavone, phenobarbital or aroclor 1254. Thereafter, the livers are harvested. As a liver of a rat weights approximately, 2 % to 4 % of the animals´ body, 200 mg to 300 mg liver can be obtained from one animal. This results in 300 μl of a 10-30 $mg_{protein}$/ml solution. This product is applied for example in the *in vitro* mammalian cell micronucleus test (OECD 487), H295R steroidogenesis assay (OECD 456), bacterial reverse mutation test (OECD 471) or Ames fluctuation test (ISO 11350). The demand for this resource varies in the different bioassays. However, it has been estimated that on average 4.5 % v./v. of this solution is applied per assay. In addition, due to the biological origin of this resource the batch-to-batch variations are high. Therefore, these error-prone characteristics increase the assay-based variations. Here, animal-free alternatives are introduced. Using suspension cell lines cultivated in a universal animal-component free, chemically-defined medium and maintained in suspension. $ewoS9^H$ (human), $ewoS9^R$ (rat) and $ewoS9^Z$ (zebra fish) represent the S9 subcellular fractions after induction of the metabolic capacity. These animal free products were compared to animal derived products and proven to be experimentally better alternatives for *in vitro* testing.

TU015

Normalization of acute toxicity data to freely dissolved concentrations provides common basis for comparison between zebrafish and rodents

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Recommendations related to reductions in the use of mammals for toxicology testing include use of all available information to avoid testing if possible. However, when testing is unavoidable, other strategies that minimize test organisms, refine study objectives to conserve animal resources, and use non-mammalian organisms as surrogate test species may be applied. One non-mammalian species that has been proposed as a potentially suitable alternative is the zebrafish embryo. However, the aqueous route of exposure commonly used in fish embryo toxicity (FET) test differs from the routine dosing methods of test

substance administration (e.g., oral, inhalation, dietary, intravenous) used in rodent assays, potentially complicating the extrapolation of results from one test species to another. In the present study a mass balance model was used to transform rodent data, commonly expressed in mass units (e.g., mg/kg) to freely dissolved concentrations in blood plasma (e.g., mg/L), providing a common basis for comparing toxicity results between rodents and zebrafish. Results of acute toxicity studies in rodents exposed by oral, inhalation or intravenous routes (i.e., LD50 values in mg/kg or LC50 values in mg/m^3) were transformed to blood plasma concentrations. The transformed toxicity data show log-linear relationships between LC50 and the octanol/water partition coefficient ($\log K_{OW}$) similar to those previously reported for aquatic test species. The present work provided evidence that rodents do not differ greatly from zebrafish in sensitivity to a wide range of neutral organic chemicals, suggesting that data from aquatic toxicity studies could potentially be used to predict acute toxicity in rodents.

TU016

A validated targeted approach for metabolic profiling of fathead minnow embryos

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Fish embryos are a popular study object because they represent a sensitive stage of development that is vulnerable to toxicant action. While the standard fish embryo toxicity (FET) test is focused on lethal parameters, the integration of sub-lethal metabolomics-based endpoints may provide more sensitivity and yield additional mechanistic information. Before this technology can be applied in FET testing, analytic method validation is required to ensure quality, reliability and consistency of future metabolomics datasets. Therefore, a methodological validation study was performed to investigate the analytical and biological reproducibility of molecular profiles of fathead minnow embryos generated by a targeted metabolomics assay based on FIA- and UPLC-MS/MS. Endogenous metabolites including amino acids, biogenic amines, acylcarnitines, hexoses, phosphor- and sphingolipids were measured in serial dilutions of quality control samples made from whole body embryo extracts to specify detection limits and identify potential ion suppression effects. Analyte recoveries and matrix effects were determined by spiking embryo extracts with analytical standard mixtures and retention time stability was assessed comparatively across different runs. Furthermore, biological variability was estimated by comparing metabolite profiles of different individuals originated from one and different clutches as well as of different developmental stages. Here we introduced and validated a targeted metabolite profiling workflow for fathead minnow embryos. Validation procedure displayed good linearity, recoveries and instrumental precision with low technical coefficients of variations. As expected biological variability was higher than technical variability but was in line with published values for fish plasma and tissues. Overall the analytical method was proven to be operational. Regarding future experimental designs standardization should be applied in terms of developmental stage control and selection of embryos from one single clutch, since these factors were shown to be major sources of variability. This work has received support from the EU/EFPIA Innovative Medicines Initiative Joint Undertaking (iPiE grant n° 115735)

TU017

Nanoparticles: how safe are they? Schmidtea mediterranea as a model organism for toxicity assessment

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Nanoparticles (NPs) are considered safe for prolonged internalization in cells. During the latest years, an increasing amount of studies indicates cytotoxic effects of different nanoparticles (NPs). On the subject of genotoxicity, carcinogenicity and stem-cell-related toxicity, conflicting results are reported. Stem cells are an important target in several applications of NPs, f.e. in stem cell tracking systems, regenerative medicine and anti-cancer drugs... New insights in the toxicity of nanoparticles on stem cells not only address these aspects, they also contribute to our knowledge on defense mechanisms of different tissues. It is generally known that the intrinsic cellular repair capacity varies with the degree of cell potency, an important matter in developmental toxicity as well as in species- or tissue-related plasticity. *Schmidtea mediterranea* is a small invertebrate with an easy accessible population of totipotent stem cells. This makes it possible to study underlying mechanisms of nanoparticle toxicity on stem cells *in vivo*, and as such link them to physiological parameters. We focus on the toxicity and biocompatibility of silver NPs (AgNPs) and silica NPs (SiNPs) on stem-cell-specific activities. Both AgNPs and SiNPs are extensively used in biomedical applications, s.a. disinfectants, implants, as drug carriers or in bio-imaging. In our *in vivo* model system, we observed the uptake of AgNPs in stem cells via transmission electron microscopy. Stem cells responded by a proliferation decrease, measured via histon H3 immunostaining. We are currently looking into the underlying cause of this decrease, as the genotoxic character of this element is still under debate. Effects on cell cycle progression and differentiation were investigated by looking into the expression of the *smedwi-1* gene and corresponding protein levels, both exclusively present in stem cells and indicative for different phases of the cell cycle. At the phenotypic level, concentration-dependent behavioral effects, including looper movements and curling, were observed. Exposure to a range of SiNPs concentrations showed no clear phenotypic or proliferation effects. This is a promising result for the further use of SiNPs, but extra research on the uptake and the effects of coating, and the influence of external parameters are needed for both NP types to confirm initial results.

TU018

The endocrine axes of fish and amphibians share common key events identified using the concept of Adverse Outcome Pathways (AOP)

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In order to be defined as endocrine disrupting chemical, a substance has to meet a number of criteria, including the induction of adverse effects, specific endocrine mode-of-action and plausible link between these. Especially the latter criterion might not always be unequivocally determined, particularly as the endocrine system consists of diverse endocrine axes, e.g. the hypothalamus-pituitary-gonadal (HPG) axis, the hypothalamus-pituitary-thyroidal (HPT-) axis, and the hypothalamus-pituitary-adrenal/interrenal (HPA/I-) axis. These axes closely interact with each other, and manipulation of one is not without effects on the other. In order to provide mechanistic understanding of these interactions, the concept of Adverse Outcome Pathway (AOP) might be used. Therefore, in this study common key events (KE) and key event relationships (KER) shared by two or more endocrine axes were identified by focusing on fish and amphibians, however, data also on mammals were considered, as most of the mechanisms of the three endocrine axes are evolutionary conserved. This literature review comprises data of ecotoxicological and toxicological studies assessing adverse apical effects, as well as of basic research on physiological processes applying molecular biological approaches. The gathered information delivers data on the interaction of individual biological elements, for example hormone/ hormone receptor interactions, gene transcription regulation, or enzymatic activity. The so identified KEs and KERs provide explanations for unexpected effects on one axis, exerted by substances suspected to act specifically on another axis. Based on the data of the literature research, eight AOPs were identified. These AOPs describe three connections between the HPG- and HPT-axes, four connections between the HPG- and HPA/I-axes, and one connection between the HPT- and HPA/I-axes. Central KEs identified across axes were altered aromatase activity, and altered expression and function of proteins 11β-Hydroxysteroid Dehydrogenase (11ß-HSD) and Steroidogenic Acute Regulatory (StAR) protein. Substance classes that act on more than one endocrine axis were for example goitrogens like the perchlorates, aromatase inhibitors like prochloraz, or chloroacetamide herbicides. Even though the developed AOPs represent just a basic structure and data gaps have to be closed at this point, this approach holds high chance for later application to evaluate the endocrine properties of substances.

TU019

In silico molecular docking for EDC toxicity prediction in the nematode Caenorhabditis elegans

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In silico molecular docking is the prediction of ligand-receptor complex structure and binding affinity and can be used for identification of molecular initiating event and toxicity. Recently, OECD developed Conceptual Framework for the Testing and Assessment of Endocrine Disrupters to provide a list of standardized test methods from *in silico* to *in vitro* and *in vivo*. Endocrine disruptive chemicals (EDCs) can affect hormonal systems by interfering with receptor signaling. In this study, aimed to develop predicting tool for toxicity of EDCs with ligand-receptor binding affinity, we screened potential and known EDCs through *in silico* molecular docking analysis and experimental validation with the nematode *Caenorhabditis elegans* (*C. elegans*) by using reproductive failure as an endpoint. First, we conducted homology modeling of *nhr-14* as estrogen receptor and *nhr-69* as androgen receptor in *C. elegans*, followed by molecular docking simulation of these nuclear hormone receptors. Next, we analyzed receptor-ligand binding affinity of selected chemicals (bisphenol A, nonylphenol, benzo[a]pyrene, fenitrothion, etc.) with their reproductive toxic potentiality in 72 h exposed wild type (*N2*), *nhr-14* and *nhr-69* mutants worms. The present study demonstrates that *in silico* molecular docking simulations of *C. elegans* nuclear hormone receptors and ligands could be used to predict chemical toxicity and screening tool for potential EDCs. Keyword:Endocrine disruptive chemicals, Toxicity prediction, Molecular docking, *Caenorhabditis elegans*

TU020

Mode of Action (MOA) assignment classifications for ecotoxicology: Overlap and consistency of available schemes

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Various structure-based classification schemes to categorize chemicals based on mode of action (MOA) have been developed for ecotoxicological risk assessment. A clear understanding of how each of these MOA schemes are devised, what information they are based on, and the limitations of each approach is critical to make a reliable use of classification or grouping outcomes for the purposes of risk assessment.The most commonly used MOA classification schemes for ecotoxicology include Verhaar and Russom (included in ASTER), both of which are used to predict acute aquatic toxicity MOA. Verhaar is a QSAR-based system that classifies chemicals into one of four classes, with a 5th "other" class, whereas Russom includes eight classifications. MOATox is a third classification scheme which classifies chemicals into six broad MOAs and 31 specific acute aquatic toxicity MOA subcategories. This work aims to evaluate and compare the ASTER, Verhaar and MOATox MOA schemes and several automated MOA assignment tools in which they are implemented (OECD QSAR Toolbox, Toxtree, ASTER) that allow users to automatically attribute the MOA of a chemical based on structural rules or presence/absence of structural moieties. In order to do so, the technical basis, applicability domains and limitations of the various classification schemes are presented. Overlapping and consistency obtained across MOA classification schemes using the different automated tools have also been analyzed, using a dataset of 426 chemicals. Inter-scheme discrepancies have been further identified and investigated to better understand their origins. Future needs for harmonization to improve consistency and reliability of acute aquatic toxicity MOA assignment are discussed. \n \n

TU021
Amiodarone and Terfenadine Induced Cardiotoxicity in Developing Zebrafish Embryos (Danio rerio)
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Drug-induced cardiotoxicity is a leading factor for drug withdrawals, and limits drug efficacy and clinical use. Therefore, new alternative animal models and methods for drug safety evaluation have been given great attention. The zebrafish has been developed and evaluated as a brilliant model for its ability to predict the toxicity of chemicals and for evaluating drug-induced cardiotoxicity. The heart of zebrafish provides a useful vertebrate cardiac and cardiovascular model with outstanding advantages, including optical accessibility and rapid development. In this study, taking advantage of transparent zebrafish embryo (48 hour post fertilised), we evaluated cardiovascular toxicity of amiodarone and terfenadine at the concentrations of 0.1, 0.5, 1.0, 10.0, 25.0, 50.0, 75.0, 100.0, 150.0, and 200.0 μM after 3 h of post exposure. Amiodarone is antiarrhythmic drug and it is used for the treatment of arrhythmia (heart rhythm disorders of the ventricles). Terfenadine is used as antihistamines to prevent allergic symptoms like sneezing, runny nose, itching, and watering of the eyes. The qualitative and quantitative cardiotoxicity was assessed using specific phenotypic endpoints: heart rate, heart rhythm, pericardial edema, and circulation abnormalities. Visual endpoints, such as lethality, edema (the presence of heart edema), abnormal body shape (including bent or misshapen caudal region of the larvae) and motility, were evaluated as general toxicity endpoints. Amiodarone and terfenadine exhibited arrhythmia, very slow blood circulation, and absence of blood circulation. It concludes that both drugs have cardiotoxic potential. Although both compounds are used for ailments in human but they exhibit cardiotoxic effect when exposed to early development. The present research recommends that cardiotoxicty effect should be checked as it is valid and reliable model for rapid prediction of chemical toxicity during drug research and development.

TU022
Examination of the relevance of Hansen Solubility Parameters to prediction of ecotoxicity and bioconcentration
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Hansen solubility parameters were developed in 1967 as a way of predicting whether a substance will dissolve in another and form a solution. They are based on the concept that like dissolves like where one substance is defined as being 'like' another if it bonds to itself in a similar way. A substance is given three parameters: Delta d: The energy from dispersion forces between molecules Delta p: The energy from dipolar intermolecular force between molecules Delta h: The energy from hydrogen bonds between molecules. These three parameters (HSP) are co-ordinates for a point in three dimensions known as the Hansen space. The values for a substance can be obtained in an automated way. It is well-known that many attempts are made to describe complex molecular behaviour in terms of a single number (such as Kow, the octanol-water partition coefficient). A weakness with this approach is that very different substances in physicochemical terms may share the same value of Kow. HSP may offer a more insightful approach, particularly for properties related to affinity of a molecule with a matrix. The purpose of the present work was to produce an initial survey of whether the somewhat simpler Hansen parameters are useful for description of bioconcentration in fish, and toxicity of substances in simple screening tests for *Daphnia* and algae. The parameters have been used by their developers to look at permeability of skin to substances. It is rather surprising that the present topic appears to not have been studied in a published form before. It should be remembered that whereas parameters such as Kow are only a surrogate for other phenomena, HSP offers a more fundamental and direct indication of affinity parameters, particularly relatable to processes such as solubility, transfer into and across cell walls and membranes, solubility in fat, adsorption to relevant surfaces such as that of soil, and related underlying science. Organisms can be considered as an organic phase in which a chemical may have higher or lower relative solubility compared to that in surrounding aqueous media. Hence bioconcentration in fish represents a case where relative solubility and transfer are relevant. Toxicity to *Daphnia* and algae may also be considered initially as a process of body burden inducing adverse effects. The present work explores the predictability of these properties and discusses the successes and limitations seen.

TU023
In vitro characterization of the toxicity of selected emerging contaminants to lake trout
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There is an ever-increasing number of chemicals including pharmaceuticals and industrial pollutants that are released into the aquatic environment, leading to the exposure of fish and other aquatic life. To date, there is limited knowledge about the potential effects and the sensitivity of native fish species in North America to these emerging chemicals. However, given the huge number of compounds that will have to be tested, there is a need to address the ethical concerns regarding the large number of animals required for current standard *in vivo* toxicity testing, particularly when working with protected and endangered species. Therefore, the aim of this present study was to investigate the potential toxicity of selected emerging chemicals of concern, namely 17a-ethinylestradiol (EE2), prozac, and silver nanoparticals (AgNPs), to lake trout (*Salvelinus namaycush*) using *in vitro* gonadal and liver explant assays. Gonadal explants were used to investigate disruption of sex-steroid hormone synthesis upon exposure to these compounds. Liver explants were used to investigate the general toxicity of these compounds as well as their effects on selected pathways including metabolism, oxidative stress and endocrine disruption. Lake trout were sampled in northern Saskatchewan, gonads and liver were excised, sliced into $1mm^3$ pieces and placed into a 24-well cell culture plate containing supplemented L-15 media. Tissue explants were exposed to increasing concentrations of forskolin, prochloraz, EE2, Prozac, or AgNPs, along with a solvent control (DMSO) for 24 hrs. Hormone concentrations in media were quantified using ELISA. Abundance of various transcripts from exposed liver explants were quantified using q-PCR. This study is part of a larger ongoing research effort that investigates the toxicity of the above compounds to four other species native to Canadian freshwater systems (rainbow trout, lake whitefish, northern pike, white sturgeon) using the here featured *in vitro* as well as early life-stage *in vivo* approaches, and which will be used to characterize the sensitivity of these species to these chemicals.

TU024
Challenges with Algae Growth Inhibition Test including Recovery based on OECD Guideline 201 with Non-Standard Algae Species
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The 200 series of OECD Guidelines for the testing of chemicals propose various species with which the testing shall be performed. Basis for this recommendations are ring tests which have shown under which specific and standard conditions the test with these algae species can be performed. One of these guidelines and maybe the most important one for the risk assessment of herbicidal acting plant protection products is the OECD 201, Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Although the proposed species covers most groups of photosynthetically active test organism such as green algae, diatoms and cyanobacteria, in some cases a broader variety of single test species within these groups have to be tested to refine the risk assessment for plant protection products. In addition to that and to be closer to the real situation in the environment, the recovery potential of the different photosynthetically active species can be investigated. The recovery potential of the different species after exposure to growth inhibiting substances is not standard for risk assessment and therefore not covered by the mentioned OECD 201. Therefore the setup needs a highly sophisticated pretesting and a very detailed plan to ensure that the resulting data give answer to the open questions. A combination of both,

non-standard algae species including a recovery step represents a special challenge for the planning and conducting personnel. During the reregistration process of a PPP we had the chance to perform two such studies. We will present the data of both tests during the exposure and the recovery phases (free of growth inhibiting substance) and discuss the difficulties of such tests, beginning with the search of appropriate species, the pretesting, the development of a proper test design, the performance of the test itself and the interpretation of the test.

TU025

Evaluation of bioaccumulation potential of several pharmaceuticals based on log D/BCF data from the standard OECD 305 protocol and the OECD 305 minimised test designed

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As per the EMA Guideline for Environmental Risk Assessment of Medicinal Products for Human Use, a fish bioconcentration study is triggered in Phase I for pharmaceuticals having a log $K_{ow} > 4.5$ to support the Persistence, Bioaccumulation and Toxicity (PBT) assessment and in Phase II, Tier A for pharmaceuticals having a log $K_{ow} > 3$. The recommended protocol for bioconcentration is OECD Test Guideline 305: Bioaccumulation in Fish, Aqueous and Dietary Exposure. Based on the standard 305 sampling schedule, approximately 200-300 fish per study may be required to determine a steady-state (BCF_{ss}) and a kinetic (BCF_k) bioconcentration factor. The current 305 guideline (2012) includes the minimised test design as described by Springer *et al* (2008). This design uses fewer sampling time points, reducing the number of animals and resources required to establish BCF values, and as per the guideline, may be used for substances in which uptake and depuration are expected to follow first order kinetics; behavior generally associated with non-ionisable organic substances. Considering the potential for fish to metabolise xenobiotics and the number of animals required for a standard BCF test, an evaluation of BCF values obtained for several pharmaceuticals was conducted to understand 1) whether existing data support the current log K_{ow} trigger of 3 for BCF testing and 2) whether the minimised test design may be considered appropriate for ionisable compounds such as pharmaceuticals. Based on the current log K_{ow} trigger and 'B' classification criteria, BCF < 1000 (US) and < 2000 (EU), the suitability of the current trigger was assessed using BCF and log K_{ow} values for approximately 45 pharmaceuticals. For those pharmaceuticals classified as 'B', a further assessment of persistence and toxicity classifications was conducted to understand the percent of pharmaceuticals within this dataset that are also classified as PBT, or vPvB. In addition, the BCF_k values obtained using standard OECD 305 test method were compared to the estimated kinetic (BCF_{km}) values determined using the reduced sampling method or Minimised Test Design, to determine if such a test design may be considered appropriate for ionisable substances. Evaluation of the data support an increase in the log K_{ow} trigger for BCF and indicate the minimised test design may be appropriate for ionisable substances such as pharmaceuticals, provided uptake and depuration follow first order kinetics.

TU026

Toxic and Activation Effects of Low-Level Radiation via Bacterial Luminescent Assay. Description in Terms of Hormesis and Threshold Models

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Effects of alpha- and beta-emitting radionuclides (americium-241 and tritium), and gamma radiation (^{137}Cs-containing particles) on luminous marine bacteria were studied under conditions of chronic low-dose irradiation (< 0.2 Gy) in aqueous media; bioluminescent intensity was used as a tested physiological parameter. The luminous bacterium is a proper tool for study the low level exposures due to simplicity and high rates of assay procedure, providing a lot of samplings under comparable conditions and, hence, a proper statistical treatment. Non-linear dose-effect dependencies were demonstrated. Three successive stages in the bioluminescent response to alpha- and beta-emitting radionuclides were found: 1 - absence of effects (stress recognition or threshold effect), 2 - activation (adaptive response), and 3 - inhibition (suppression of physiological function, i.e. radiation toxicity). Gamma irradiation demonstrated only stages 1 and 3, while the bioluminescence activation stage (2) was not found. The bacterial response was found to be independent on activity concentrations of radionuclides or dose rates of gamma-radiation. The nonlinear dose-effect dependencies of ionizing radiation with activation phenomenon included (stage 2), were ascribed to the "hormesis" phenomenon. The effects of gamma-radiation were described in terms of "threshold" toxicity model. Experiments with tritiated water and tritium-labeled polyethylene films (liquid and solid courses of beta-particles, respectively) showed that activation of the intracellular bioluminescence process can take place without penetration of tritium into the cells. Sequence analysis did not reveal mutations in bacterial DNA under conditions of the experiments. The results give preference to a "non-genomic" mechanism of bioluminescence activation. Probably, the activation effects result from ionization of aqueous media followed by the intensification of cellular membrane processes. Biological role of reactive oxygen species, secondary products of radioactive decay, is discussed. Key words: bacterial luminescent assay, low-dose radiation toxicity, hormesis model, threshold model.

TU027

Use of an integrated testing strategy to fill data gaps for environmental risk assessment of iso-alcohols

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Environmental risk assessments require quality data to provide defensible environmental quality benchmarks. Quantitative Structure Activity Relationship (QSAR) endpoint estimates are often appropriate for alcohols with a very strong correlation to aquatic toxicity test data. However, QSAR estimates require comprehensive justification to demonstrate applicability, and still may not fully meet regulatory requirements, leading to extensive long-term toxicity testing. Here, limited, strategic environmental testing was used to support QSAR predictions, thereby reducing animal testing while still meeting regulatory requirements. Aquatic toxicity testing with algae, daphnids and fathead minnows (OECD 201, ISO 20665 and OECD 210) was performed with isooctanol and isoundecanol. The study objective was to employ a testing program consisting of long-term fish (limit test), invertebrate and algal toxicity tests to demonstrate that QSAR estimations accurately predict aquatic effects from long-term continuous exposure to these substances, further supporting the use of QSAR models across a range of iso-alcohols. The data demonstrate that the QSAR model employed accurately characterized the hazard of iso-alcohols and is protective of these endpoints. Moreover, this combined information, by demonstrating a regular and predictable pattern of toxicity amongst these substances, further justifies read-across between substances for other endpoints (such as bioaccumulation) and supports efficient use of data for general purpose risk assessments.

TU028

OECD 201 - Comparison of Algae Growth Inhibition Reference Studies Conducted in a Conventional and Closed System

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Some of the substances which have to be registered under REACh are volatile. The design of ecotoxicological studies such as the fish toxicity test (OECD 203), Daphnia immobilisation test (OECD 202) and Algae growth inhibition test (OECD 201) is different from standard cases as indicated in the OECD 23 and ISO 14442 Guidance documents on aquatic toxicity testing of difficult substances and mixtures. Especially for the algae growth inhibition test following the OECD 201 the differences are obvious: without the CO_2 – supply from the surrounding air the algae cannot grow. Therefore these algae need other carbon sources, e.g. $NaHCO_3$. Additionally to keep the pH of the test media as constant as possible, HEPES-buffer has to be added to the test water. The consequences of these special conditions for the algal growth have to be checked by performing controls. Not much literature is available which compares the growth of algae under open and closed conditions or compares the possible differences in sensitivity of the algae to substances between algae which grow under normal condition and in a closed system. To have a reliable data set we conducted studies with the same algae strain and under conventional and closed system conditions to investigate these differences. The results show that there are differences in sensitivity of the algae when the growth inhibition between the studies conducted in the closed (ECr_{50} = around 0.7 mg potassium dichromate/L) and the open (ECr_{50} = around 0.9 mg potassium dichromate /L) system are compared, but the differences are, in most cases, negligible for the risk assessment. In certain cases, when the toxicity EC_{50} values are closed the trigger values, e.g. 1.0 mg/L, the difference can be critical for the classification of the substance.

TU029

Development of a zebrafish embryo test for environmental risk assessment of pharmaceuticals with different endocrine disrupting modes of actions

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Pharmaceutical companies are obligated to perform an environmental risk assessment for each pharmaceutical that is launched on the market. The current tests for potential endocrine disrupting compounds are not consistent with the 3R principle. Therefore, the goal of this study is to develop a zebrafish embryo test, which is not considered an animal test according to the European regulation on the use of laboratory animals, and which is capable of detecting and discriminating among 5 endocrine disrupting modes of action (MOAs). The MOAs that are studied are estrogen receptor (ER) agonism and antagonism, androgen receptor (AR) agonism and antagonism and aromatase inhibition. As a first stage of test development, one pharmaceutical per MOA was investigated to map differences among the 5 compounds in zebrafish embryos on morphological, physiological and molecular levels. 17α-ethinylestradiol (EE2) was chosen as ER agonist, fulvestrant as antagonist. 17β-trenbolone and flutamide were respectively chosen as the androgen receptor (AR) agonist and antagonist and letrozole was the aromatase inhibitor. Zebrafish embryos were exposed to one of the pharmaceuticals or to dimethyl sulfoxide (vehicle control) and monitored until 120 hours post fertilization (hpf). During these 5 days multiple endpoints were evaluated. Survival was scored daily, heart rate at 24 hpf, hatching from 48 hpf and morphological endpoints and swimming activity at 120 hpf. Both agonists showed multiple morphological and physiological effects, the antagonists showed no effects or only mortality and the aromatase inhibitor only showed physiological effects. The differentiation potential of all those endpoints was however not high enough to differentiate the 5 MoAs. To increase this potential a transgenic zebrafish line (*5xERE-GFP*, obtained from the Carnegie Institution of Washington) was used. It expresses GFP upon ER activation and fluorescent intensity was measured at 120 hpf. Even at low concentrations embryos exposed to EE2 caused a significantly higher fluorescent signal compared to controls. Embryos exposed to the non-aromatizable androgen, 17β-trenbolone also gave a significant signal at very high concentrations, suggesting that another pathway is triggered to elevate estrogen concentrations. The other 3 compounds caused no significant signals as expected. In the future other endpoints will be added in order to find a combination of endpoints that can be used to discriminate the MoAs.

TU030

Development and need of a new marine cyanobacteria test for the ecotoxicological risk assessment of antibiotics applied in the marine environment

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An increasing amount of pharmaceutical products in waterbodies all over the world is been detected. Antibiotics play a special role here. In addition to the high amount of medication, antibiotics are as well an ecologically important pharmaceutical group due to the specific activity against prokaryotes and a possible resistance formation in the environment. Especially in oligotrophic marine waters, phototrophic prokaryotes together with small eukaryotic algae make up a large percentage of the total primary production. The gramnegative phototrophic prokaryotes including the cyanobacteria and thus have prokaryotic target structures of antibiotics. For the approval of pharmaceutical products, no prokaryotic test system in the marine field of application is considered in the risk assessment so far. The potential effects of antibiotics especially for aqua cultural use on the primary production and the Nitrogen fixation are unknown. To investigate the effects on marine cyanobacteria a marine cyanobacteria test was developed. As test organisms two marine species *Anabaena spec.* and *Synechocystis spec.* were selected and cultivated in the laboratory. The cultivation method was transferred to 24-well microtiter plates and optimized. The test was carried out on a 24-well microtiter plate. In the test the percent inhibition of the growth rate (cell number) compared to the negative control is determined over a period of 72 hours. Marine cyanobacteria react more sensitive to the tested antibiotics than the marine eukaryotic algae *Phaeodactylum tricornutum* (Bacillariophyceae, DIN EN ISO 10253). The results show effects on non-target organisms in relevant environmental concentrations (μg L^{-1}). The investigations are carried out within the PharmCycle project with the overall aim to reduce pharmaceuticals in surface water, ground water and drinking water. PharmCycle has the goal to optimize the ecotoxicological risk assessment, to reduce pharmaceuticals in waste water treatment and to develop sustainable biotechnological and chemical produced antibiotics (green pharmacy). The project is conducted in several cycles, in which priority antibiotics and sustainable antibiotics are examined.

TU031

Evaluation of skin sensitisation potential of 4-tert-butylphenol and 4-(benzyloxy)phenol using in vitro skin sensitisation assays

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Contact dermatitis is an inflammation of the skin characterised by redness, itching, blistering and in chronic cases, flaking of scales of skin, resulting from exposure of the skin to substances in the environment. Various *in vitro* assays like Direct Peptide Reactivity Assay (DPRA), Amino acid Derivative Reactivity Assay (ADRA), KeratinoSen, USens, hClat have been developed and accepted for the prediction of skin sensitisation potential. p-tertiary-butylphenol formaldehyde resins (PTBP-FR) are widely used as binders in industry and in numerous materials of everyday use, such as glues, adhesives, or inks. The adhesive property of these resins is also exploited for preparation of '**bindi**' adhesives (adhesives used at the back of decorative patch, usually put on the middle of the forehead by girls/ women in the sub-Indian continent). The objective of the present study was to determine the sensitizing potential of 4-tert-butylphenol & 4-(Benzyloxy)phenol (constituents of PTBP-Formaldehyde resins) using *in chemico*, *in vitro, and in silico* skin sensitisation assays. The *in chemico* skin sensitisation assays employed were DPRA & ADRA. The mean percent depletion of cysteine and lysine for 4-tert-butyl phenol and 4-(Benzyloxy)phenol were 2.40 and 18.85 respectively in DPRA assay. While, the mean percent depletion for 4-tert-butyl phenol and 4-(Benzyloxy)phenol were 1.36 and 34.53 respectively in ADRA assay. From the results of the *in chemico* assays, 4-tert-butylphenol was found to be a non-sensitiser while, 4-(Benzyloxy)phenol was identified as a sensitiser. In ARE-Nrf2 Luciferase Test method (i.e, KeratinoSens assay), calculated $EC_{1.5}$ values of 4-tert-butylphenol & 4-(Benzyloxy)phenol were found to be 3 μM & 24 μM respectively. Thus, from the results of KeratinoSens assay, both 4-tert-butylphenol & 4-(Benzyloxy)phenol were found to be sensitisers. In U-Sens assay, the endpoint is the calculation of Simulation Index (SI) which if found to be > 1.5 is considered as a sensitiser. Both the chemicals showed SI > 1.5. Hence, these chemicals were predicted as sensitisers. Results of QSAR also suggest these chemicals to be sensitisers. Thus, on the basis of results of all assays and using the '3 of 5' weight of evidence approach, 4-tert-butylphenol & 4-(Benzyloxy)phenol were concluded as sensitisers.

TU032

Chemoassay Profiling to Characterize the Skin Sensitization Potential and Potency of Organic Electrophiles

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Allergic contact dermatitis is a worldwide health disease and is initiated by sensitizing the immune system through dermal contact with a skin protein covalently bound to an electrophilic xenobiotic. Possible sources of respective organic electrophiles include care products, cosmetics and industrial products. At present, REACH recommends the murine local lymph node assay (LLNA) as method of choice for evaluating the skin sensitization potential of chemical substances, calling for viable alternatives to replace this animal test. In this context, chemoassays have been recognizes as promissing non-animal alternatives to identify potential skin sensitizers through characterizing electrophilic reactivity. So far, only full-kinetic and mechanism specific approaches inform about the sensitization potency. For volatile electrophiles, chemoassays tend to overpredict their LLNA potency because these test compounds significantly evaporate from the murine skin, and thus are less available in vivo to react with nucleophiles of skin proteins (as compared to chemoassay conditions). These volatile compounds are typically excluded from respecitve reactivity-based prediction models. Moreover, for imine formers as aldehydes no chemoassay-based models for predicting the LLNA-type sensitization potency are available. The present communication demonstrates for Michael-acceptor carbonyls how LLNA potency of volatile electrophiles can be predicted through combining the chemoassay thiol reactivity[1] with the concept of chemoavailability.[2] The latter enables one to specifiy the conditions under which a given electrophilic reactivity triggers a reactive toxicity pathway in the physiological context of interest. Moreover, a chemoassay-based model to predict the LLNA-type sensitization potency of imine formers is presented, and thus extends chemoassays toward this class of potential sensitizers. The authors thank the EU-funded project OSIRIS (contract no. GOCE-CT-2007-037017) and the BMBF-funded project ProHapTox (FKZ 031A422A and 031A422B) for financial support. [1] Böhme A, Thaens D, Paschke A, Schüürmann G 2009. Kinetic glutathione chemoassay to quantify thiol reactivity of organic electrophiles-application to \nα,β-unsaturated ketones, acrylates, and propiolates. *Chem. Res. Tox.* 22: 742-750. [2] Böhme A, Laqua A, Schüürmann G 2016. Chemoavailability of organic electrophiles – Impact of hydrophobicity and reactivity on their aquatic excess toxicity. *Chem. Res. Toxicol.* 29: 952-962.

TU033

Using a Database of Historical Control Avian Reproductive Performance to Enhance Evaluation of Agrichemical Product Toxicity

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Avian reproduction studies with northern bobwhite *(Colinus virginianus)* and mallard *(Anas platyrhynchos)* have been conducted for nearly 40 years. The information collected from these gamebird species has been a primary part of the OECD and EPA regulatory program in evaluating agrichemical products and making environmental risk assessments. Control data from these two species have been collected and maintained since the late 1970's by Wildlife International (now operating as a division of EAG Laboratories). The databases compile values for reproductive parameters measured from birds used in each control group as part of each reproduction study conducted at the lab in Easton, Maryland, USA. The database summarizes mean numbers of eggs laid, fertility of the eggs, normal development including viability and survival of the embryos, hatchability, offspring survival, egg shell thickness as well as weights of offspring and 14-day old survivors. Data compiled in these records are becoming more critical in supporting evaluation of reproduction study results. There is complexity in making meaningful decisions about the toxicity of agrichemical products. When 'wild type' birds are used we can expect inherent differences in performance and that occasional anomalies will occur. Rather than repeating multiple tests or increasing the number of birds tested, databases, such as this one, can help risk assessors interpret the validity of differences observed in the range of reproductive parameters evaluated. As the use and power of statistical programs such as CETIS® and TOXRAT® improve our ability to evaluate data and look at trends there is a need to balance these statistical evaluations with the integrity of the scientific processes observed in these toxicology tests. Moving forward a shared database system can be a valuable tool in improving interpretation of study results and evaluating mechanistic and population level effects. Maintaining a historical control database of avian reproduction studies will help risk assessors interpret the validity of differences observed in the range of reproductive parameters evaluated.

TU034

Aldehydes, epoxides and carboxylic acids: what do they really do to aquatic organisms?

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Contrarily to narcosis or specific reactivity with endogenous molecules (receptor agonists or antagonists), toxicity due to unspecific reactivity has been less studied. According to the literature, unspecific reacting compounds are often around ten times more toxic than baseline toxicity, but with greater variability. It might be expected that the toxicity of such compounds won't be predicted accurately using just hydrophobicity descriptors although in fact hydrophobicity is still a good descriptor. Nevertheless, a better understanding of the mechanism(s) of action of this class of compounds is required. In this work we compared the acute aquatic toxicity of aldehydes, epoxides and carboxylic acids with the baseline toxicity. Simple aliphatic epoxides or aldehydes can both be classified as hard electrophiles. Therefore we expect them to act through the same mechanism of toxicity and to be more toxic than baseline toxicity. Indeed, we observed that fish and daphnid toxicity data for those substances seem to follow the same relationship where hydrophobicity is still the main descriptor. As evidenced in the literature, the mechanism of action of aldehydes is the reaction of those compounds with amino residues of proteins. Epoxides also react with nucleophilic protein residues, like amino and sulfhydryl groups. Since amino and sulfhydryl groups are polar and accessible from the aqueous medium, the substances can only react if there are enough chemicals partitioning to the cytosol (plus extracellular medium for pluricellular organisms). At high hydrophobicity, both aldehyde and epoxide toxicity approaches baseline toxicity for fish and daphnid, probably because these substances remain quasi-exclusively in the membranes due to their high partition coefficient, and strongly exert a narcotic effect. Regarding carboxylic acids, no dependency to hydrophobicity was highlighted as opposed to all other groups (reactive or not). On the contrary we observed an apparent constant toxicity whatever hydrophobicity of the acid considered. We assume that their probable mechanism of toxicity is explained by releasing protons in the cytosol thus leading to acidification of extracellular medium. Indeed it is expected that only the non-ionic, protonated form of acids could cross the membrane by passive diffusion.

TU035

SETAC Animal Alternatives Interest Group

A. Lillicrap, NIVA Norwegian Institute for Water Research

Interplay between nutritional factors and chemical toxicity (P)

TU036

The role of nutritional status on tissue contaminant concentrations

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Biomonitoring of pesticides and contaminants in higher vertebrates commonly involves analysing concentrations in body tissues. The liver is usually the organ of choice because it bioaccumulates high levels of many contaminants and is often the target organ. However, the liver is physiologically dynamic and total liver mass varies with body condition—for example starvation induces liver wastage. This can affect the measurable contaminant concentration and obfuscate temporal and spatial trends in exposure. The aim of the present study was to examine the extent to which nutritional status affects bioaccumulation of contaminants in sentinel predatory bird species. We compared concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyls (PBDEs), total mercury (Hg) and second generation anticoagulant rodenticides (SGARs) in livers collected from the carcasses of starved and non-starved predatory birds. These carcasses had been submitted to the UK's Predatory Bird Monitoring Scheme (PBMS; http://pbms.ceh.ac.uk/). We found that liver concentrations of lipophilic compounds such as PCBs and PBDEs were up to an order of magnitude higher in starved than non-starved sparrowhawks (*Accipiter nisus*) whereas elevation of liver Hg associated with starvation was only 2-3 fold. In contrast starvation appears to have no effect on the liver concentrations of SGARs accumulated by barn owls (*Tyto alba*). The effect of nutritional condition on liver residues most likely depends on the physico-chemical properties of the compound and the way that it's stored in the liver. Starvation results in remobilization of lipophilic compounds from fat depots and other lipid-rich tissues and subsequent sequestration in the liver, resulting in overall marked elevation of liver concentrations [which are reported on a per weight basis]. Starvation-induced elevation of Hg residues in the liver is broadly in proportion to the extent of liver wastage and may largely reflect retention of residues even as liver mass is lost. The lack of impact of starvation on liver SGAR concentrations may be because high-affinity SGAR binding sites are lost as the liver decreases in mass. Understanding how liver residues of different compounds are affected by starvation is important for: (i) disentangling the "physiological noise" in data collected to detect temporal and spatial trends in bioaccumulation, and (ii) determining the impact of starvation on toxicity.

TU037

Development of a refined zebrafish feeding trial to evaluate food safety

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Before a *novel food component* can be introduced, a profound *safety evaluation* is required which generally includes a rodent feeding trial to assess potential toxicity. The development of a *zebrafish feeding trial* would advance existing testing strategies by reducing the cost, by replacing the use of mammals by a lower vertebrate species, and by facilitating reproductive and transgenerational studies. When evaluating novel components, part of the food needs to be substituted possibly interfering with nutritional requirements. Therefore, the extent of component substitution tolerable for the zebrafish metabolism should be assessed prior to the evaluation trial. We determined *the maximum tolerable percentage of maize* for adult zebrafish, as maize is a well-known food product for human consumption, but not commonly used in fish feed. Fish were fed for 4 weeks with 6 experimental feeds ranging from 0 to 25% of maize. Growth slightly (2.5%) decreased when fish were fed with 0% or 25% of maize. The hepatosomatic index (% liver weight relative to the total body weight) of males significantly increased when fed with 20% or 25% of maize. Feed digestibility analysis showed a decrease in carbohydrate uptake when fish were fed with an increasing percentage of maize substitution. Based on these results, we selected 15% maize as the maximum tolerable percentage for adult zebrafish. Furthermore, when novel food components are evaluated over multiple generations, the *developing animal model* should receive this component as early as possible during development. Therefore, the next phase of our study was to define the maximum tolerable percentage of maize for *zebrafish larvae*. The *ex utero* development allows us to start feeding with the experimental diets at an early age. Larvae were raised with the experimental diets (0 to 25% of maize) and the relative condition factor, length, general morphology and swimming behaviour were evaluated. In addition, transcription levels of genes encoding enzymes known to be involved in the digestive system (e.g. trypsin) were analysed at different time points during development. We suggest that our approach of determining component substitution rates for adults and, in case of a transgenerational evaluation, for early life stages could be a valuable asset to food safety evaluation trials.

TU038

Fatty Acid Characterisation of Nile Tilapia (Oreochromis Niloticus) in Fresh and Brackish Water

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Fish is mostly consumed because of its superior lipid and protein content. Fatty acid composition of a fish has so much to say about the nutritional composition of the said fish. Fatty acids are basically categorized as saturated and unsaturated; with the unsaturated further categorized as mono unsaturated and polyunsaturated (omega 3, 6 and 9). There are variations in these fatty acid composition in fish and these has been perceived to be along the diet, culture medium, location, size, sex, season, habitat of the fish. However one of the most important basis for variation is the habitat or the environment of the fish. The major habitats are fresh, brackish and marine water body and these have different composition of substances. The aim of this study therefore is to evaluate these variations in fatty acid composition if there is any and rightly infer afterwards. Samples of captured brackish and fresh water of Nile tilapia were obtained from Igbo-Olomi lagoon of Lagos State and Eleyele river of Oyo State respectively. Lipids in the muscles of the wild Nile tilapia were extracted using the Bligh & Dyer method of lipid extraction. Extracted lipids were then analyzed by gas chromatography to determine the composition and relative abundance of the fatty acids present. The results showed that fatty acids were high in both brackish and fresh water Nile tilapia with total composition of 99.99% and 99.97% respectively. A total of twenty three (23) different fatty acids were identified in both fish species. The percentage of total saturated fatty acids (SFA) was higher in the muscles of the fresh water specie (26.72%) than in the muscles of the brackish specie (24.16%). The percentage of total mono unsaturated fatty acids (MUFA) was however higher in the muscle of brackish Nile tilapia (69.56%) than in the muscle of the freshwater tilapia (65.35%). The poly unsaturated fatty acids (PUFA) were 6.27% and 7.90% in the brackish and freshwater tilapia respectively. The brackish specie however had more omega-3 (PUFA) than the wild ones (2.54% to 1.93% respectively). Both species contain essential fatty acids for promoting good health and prevention of cardio-vascular diseases in humans. **KEYWORDS: Fatty Acid, Nile Tilapia, Brackish and Fresh Water.**

TU039
Direct effects of radiation on the biochemical composition of two primary producers
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The effects of contaminants on aquatic species are commonly derived from single species toxicity tests, where organisms are exposed to dissolved contaminants. However, contaminants in general, and ionising radiation specifically, have been shown to affect food quality constituents, such as elemental ratios, as well as fatty acid and amino acid composition of primary producers. These changes in food quality parameters may indirectly influence or alter the direct effect of contaminants on a primary consumer, which single species toxicity tests do not take into account. Ecological studies have shown that both somatic growth and reproduction are linked to these food quality constituents. This study experimentally investigated the effects of ionising gamma radiation on food quality parameters of two freshwater microalgae species (*Raphidocelis subcapitata* and *Eustigmatos* sp.), which were exposed to two acute doses (0, 5 and 25 Gy) and also to three chronic dose rates (72, 34, 12.5, 0 mGy/h). Nutrient ratios (C, N, P), total protein content and 70 different metabolites (including amino acids, fatty acids, sugars, carbohydrates, sugar alcohols etc.) were measured at eight time points after the acute exposure and at two time points during the chronic exposure. Preliminary results indicated a general increase in nutrient ratios and protein content of *R. subcapitata* over time and an interactive effect of treatment and time was found in total protein content. The two species were strikingly different in their metabolite composition and the metabolite composition changed in both species over time. This study will provide important insights into changes in food quality parameters of phytoplankton species in response to ionising radiation. Such changes may help to explain why zooplankton grazers have previously been shown to consume more irradiated than non-irradiated phytoplankton.

Marine and freshwater ecotoxicology (P)

TU040
Influence of abiotic and biotic parameters on eco-physiological responses across freshwater-marine continuum as a function of salinity and metallic contaminant
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According to the potential influence of global climate changes, salinity changes on aquatic ecosystems represent tremendous challenges for the worldwide societies. Different sources of salinity changes (natural or anthropogenic) can modify the salt ratio from rivers and streams to estuarine and coastal areas. Two bivalves *Corbicula fluminea* and *Scrobicularia plana* have been selected because of their large tolerance of salinity variation allowing to study biological effects on the whole spectrum of salinity from fresh to marine waters respectively. The aim was to study the impact of a salinity stress at physiological, biochemical and behavioral levels by exposing both species to a salinity close to their limit range of tolerance, 15 practical salinity unit (psu), and at their field salinity (1.5 psu and 30 psu for *C. fluminea* and *S. plana* respectively). Two approaches have been conducted, firstly in presence or absence of food during 2 and 7 days of exposure and secondly in presence of food and different ceria nanomaterials during 7, 14, 21 and 28 days of exposure. Negative cross impacts of hyper saline condition for *C. fluminea* (15 psu) and hypo saline condition for *S. plana* (15 psu) and ceria nanomaterials have been measured at biochemical, physiological and behavioral levels. At sub-individual and individual levels, structural and energetic parameters and behavioral impairments seemed to be suitable biomarkers to assess salinity stress on *C. fluminea* and *S.plana.* After exposure to 15 psu for both organisms, fitness modifications could appeared, and may participate in endangering populations. Nevertheless, after longer exposure time (28d), different biological responses have been observed at both sub-individual and individual levels.

TU041
Native (Mytilus galloprovincialis) versus non-indigenous (Xenostrobus securis) mytilids in Biscay Bay: Different sensitivity of biological responses against environmental pollution
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The Nerbioi-Ibaizabal (Bilbao) estuary has historically been a chronically polluted place. Despite it has recently demonstrated some signs of ecological status recovery; the newly achieved equilibrium may include some undesirable aftereffects, such as biological invasion flourish. The Australasian mussel *Xenostrobus securis* is an alien species that has consistently been reported in heavily anthropized European estuarines in the last decade. *X. securis* coinhabits intertidal beds with native *Mytilus galloprovincialis*, often confidently targeted in Biomonitoring programs. Both species can be readily discriminated morphologically and anatomically although molecular markers (i.e. Glu 5') have been successfully applied to discriminate them. The main objectives of the present work were to ascertain the sensitivity of both species to a model contaminant (i.e. Cd) and understand the implications of two differing exposure scenarios (estuarine, marine) onto sublethal biological responses. Mussels (2-4 cm) *X. securis* and *M. galloprovincialis* were collected in the outer reach of the Nerbioi-Ibaizabal estuary (Arriluze) in May 2016, salinity being 28 psu, acclimated to laboratory conditions (5 d) to two salinity regimes (25 and 33 psu, T=16ºC) and exposed to Cd (0, 0.1 mg Cd/L) for 5 d. Whole mussels were processed for histology to determine histopathological alterations (tissue level biomarkers) and assess gametogenic development. Cd distribution and accumulation in target organs (digestive gland, gills, gonad, mantle) were studied by chemical and histochemical analysis (Autometallography). Tissue-level responses were seemingly species specific. Hence, the majority of the effects in *Mytilus* occurred faster and were of higher intensity than those in *Xenostrobus*. The implications of these responses will be discussed in both exposure scenarios. Seasonality could, to some degree, modulate short-term responses according to the asynchronic gametogenic development of both species, so further seasonal research is envisaged to understand biological response of *X. securis*. The present study raises awareness of the implications of a mixed sample of both species (*Xenostrobus* and *Mytilus*) in biomonotoring programs. A careful review of specimens is encouraged to discriminate both species and to give disgrated results in ecologically fragile estuaries concerning *X. securis* invasion. Acknowledgements: UPV/EHU (UFI 11/37); Basque Gov CRG (IT810-B and fellowship to EGU)

TU042
What fish have to face - Effects of anthropogenic pollution on fish health in a representative watercourse in a densely populated region of Central Europe
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The Nidda and its tributaries Horloff and Usa make up a highly anthropogenically influenced river system in the Frankfurt area, southern Hesse, Germany. Municipal wastewater and industrial pollution, as well as diffuse discharges of agricultural origin are considered impacting ecosystem integrity most notably in a negative way. Therefore, the Nidda river system is considered to be a characteristic example of medium-sized European watercourses. The *NiddaMan* project (abbrev. for *Nidda Man*agement) is investigating the *status quo* of the Nidda system on various levels. Those investigations range from i.a. biological testing and chemical screenings to modelling mass transfers and run-offs, all accompanied by socioeconomic analyses. With the final goal of *NiddaMan* not only being able to evaluate the system's ecological health but also intending a position to propose actions that might be taken to improve the condition of the river system, the project are in close contact with local authorities. The results presented here focus on parts of the biological analyses including: (i) 96 h embryo tests with *Danio rerio* investigating developmental toxicity, (ii) histopathology of actively monitored rainbow trout [*Oncorhynchus mykiss*] to assess subacute as well as of passively monitored local fish species (chub [*Squalius cephalus*], loach [*Barbatula barbatula*], gudgeon [*Gobio gobio*] and brown trout [*Salmo trutta fario*]) to detect chronic effects, (iii) biomarkers of effect to assess hormonal disruption (vitellogenin induction), genotoxicity (micronucleus test) and dioxin like effects (EROD assay) in monitored fish.

TU043
Incorporating avoidance and recolonization concepts in ecotoxicological risk assessment by using a non-forced exposure system
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Traditional laboratory aquatic ecotoxicity assays aim to assess the potential toxicity of a natural sample or contaminants on organisms, on which a given response is measured. Organisms are passively and mandatorily exposed to contamination with no possibility to avoid it. This approach, here called forced exposure, allows an obvious concentration-response relationship is derived; although we cannot neglect such direct effects, under natural conditions, mobile organisms can detect contamination and move to more favourable areas, avoiding suffering the toxic effects caused by continuous exposure. In this new scenario, no effect is expected to occur on individuals; therefore, measuring effects down to individual level lacks ecological relevance. When organisms respond to contamination moving towards

undisturbed habitats, biological effects will be sensed firstly and more intensely on ecosystems as part (or the entire) population may abandon the disturbed habitat. Therefore, the contamination will affect the spatial distribution of the organisms and the processes of habitat selection (avoidance and recolonization). Secondly, the neighbouring undisturbed habitat, used by organisms as escape area, can be indirectly stressed due to the overpopulation of avoiders. This scenario is hard to be assessed in a forced exposure system. Therefore, a free-choice, multi-compartmented, nor-forced exposure system has been proposed as additional tool able to predict how contamination can affect both avoidance and recolonization responses. By using the non-forced exposure system, it is possible to assess the biological effects caused by a gradient or even patchy contamination up to the ecosystem level. Finally, the non-forced exposure system allows evaluating the environmental fragmentation caused by chemical barrier that contamination can create, blocking the free displacement among habitats. The present study brings a novel tool for ecotoxicity assays in which organisms are not forcedly exposed to contamination, so that avoidance and recolonization responses are added to the ecological risk assessment. The theoretical basis, the operational advantages and the ecological relevance of this approach are discussed. Our experience with the non-forced exposure system has shown that this approach can provide important information about the role of the contaminants as habitat disturbers even when individual toxic effects are not detected.

TU044

Impact of the antidiabetic drug metformin and the artificial sweetener sucralose on brown trout

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In the last decades, the number of patients suffering from life style diseases like diabetes type 2 or obesity is increasing. Consequently, there is a rising consumption of substances used for treating these diseases, as e.g. the antidiabetic drug metformin and the artificial sweetener sucralose. The insufficient removal in wastewater treatment plants results in relatively high concentrations of these compounds in surface waters. However, possible effects of metformin and sucralose in aquatic organisms are far from being understood. The aim of this study is therefore to investigate influences of metformin and sucralose on different metabolic pathways and behaviour in different life stages of brown trout (*Salmo trutta* f. *fario*). Two experiments were conducted: firstly, eggs of brown trout in the eyed ova stage were continuously exposed to different metformin concentrations (0, 1, 10, 100, 1000 μ/L). The experiment was conducted at 7°C and 11°C regarding possible interactions of chemical toxicity and temperature. To show influences on the embryo development, mortality, hatching success, and time of hatch were recorded. Tissue samples were taken at the end of the sac-fry stage. Secondly, juvenile trout (age: 8 month) were exposed for 23 d at 7°C to different metformin and sucralose concentrations (0, 10, 1000 μg/L metf., 1000 μg/L sucr., 1000 μg/L metf. + 1000 μg/L sucr.). In both experiments, several endpoints indicating fish health were investigated, including glycogen storage and histological condition of the liver, the blood sugar level and alterations in the level of the stress protein Hsp70. Additionally, animal behaviour (swimming velocity, predator-prey-interaction) was investigated. Metformin did influence neither hatching rate nor heart beat rate in brown trout larvae. In both experiments, the blood sugar level was tendentially lower in metformin-exposed fish (in 1000 μg/l metformin) compared to the control and there was no influence of metformin on the mortality of trout. Histological data and results of stress protein analyses will be presented (analyses not yet finished). This work is part of the project Eff-Net (Effect Network in Water Research) funded by the Wassernetzwerk Baden-Württemberg. By a multi-disciplinary approach, antidiabetics, artificial sweeteners and antidepressants as well as their metabolites and transformation products are studied from the mode of action at the molecular level up to the effects at the community level.

TU045

Marine Species Testing

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Discussion of marine species testing for the risk assessment of pesticides is increasing. The US EPA already provides some test guidelines. Many of these guidelines have been in a draft status for several years. Furthermore, many universities are working on different questions regarding the effects of pollutants to marine ecosystems. The strongest influence on marine systems is expected at the coastal zones, where not only the highest concentrations of anthropogenic substances are present but also the biggest abundance and diversity of species exist. A focus on estuarine species is likely since they are the link between river and marine systems. But the question remains if ecotoxicology testing with marine organisms is comparable with tests performed with fresh water species and whether there is a difference between fresh water ecosystems and marine ecosystems for risk assessment. The wide range of species presents in marine ecosystems makes it difficult to determine which species are representative for the marine ecosystem as a whole. Furthermore, due to a lack of a complete taxa of the insects, the difficulty for a comparison to fresh water systems is exacerbated. Since the role of insects and crustacean is comparable, a focus should be placed on crustaceans for marine testing. Potential species will be selected with the focus on the comparability to the existing guidelines. An emphasis will be placed on species which are able to live in salt and fresh water systems in order to understand if the change of habitat has an influence on the sensitivity to pollutants.

TU046

Acetylcholinesterase activity and degree of lipid peroxidation as biomarkers of the toxic effects of pesticide DDVP (2,2, -diclorovinyl-phosphate) on Artemia franciscana

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Dichlorvos is a highly toxic insecticide (EPA, 2000), this compound is used for pest control in fish farming. Ecotoxicological studies have been developed with this compound in a variety of species and has been shown to aquatic organisms, cause significant effects deleterious as: decreased respiratory rate, alterations in the activity of important enzymes, in addition to inhibiting the feed rate and cause haematological alterations. Despite this, there are few studies evaluating its toxic effect on marine organisms, for this reason the objective of this study was to determine its toxicity and to evaluate 2 biomarkers: the activity of the enzyme Acetylcholinesterase (AchE) and the degree of lipid peroxidation caused by the acute and sublethal exposure of the DDVP in *A. franciscana*. Bioassays were performed where 5 concentrations of the insecticide (50.84, 5.084, 0.5084, 0.05084, 0.005084 mg L^{-1} plus a negative control) were tested on *A. franciscana* nauplii obtained from cysts hatching and adults. At 48 hours (nauplii) y 96 hours (adults) of exposure, LC_{50} (lethal concentration 50) was determined and samples of living organisms (20 per treatment) were taken to determine the level of lipoperoxidation, by means of the evaluation of Tbars (Buege and Aust, 1978) and the activity of the enzyme AchE as an indicator of neurotoxic effects (Ellman et al., 1961). In the lethality tests, *A. franciscana* was more sensitive to DDVP compared to other species such as Japanese shrimp (*P. japonicus*), brown shrimp (*Litopanaeus duorarum*) and American lobster larvae (*Homarus gamarus*). In the evaluation of biomarkers, significant differences were obtained between the control and organisms exposed to the pesticide ($p < 0.05$), the nauplii were more sensitive than the adults. In the determination of the activity of the enzyme AchE it was observed that the increase of the pesticide concentration decreased the activity of this enzyme. The percentage inhibition of AchE activity varied from 8 to 43%. The degree of lipid peroxidation ranged from 8.5 to 73.3 nM Tbars mg protein^{-1} and was 1.2 to 11 times higher, compared to the control group (6.59 nM MDA mg protein^{-1}). The results of this study showed that DDVP has a neurotoxic and oxidative effect in sublethal concentrations in *Artemia franciscana*.

TU047

The aquatic life criteria for Endosulfan in the Yangtze River Delta Region of China

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With the rapid increase in pesticide production and use in China, there has been growing concern about the potential impact of pesticide exposure on the surface water and aquatic organism in the country. Aquatic organism criteria are the scientific basis to formulate water quality standard, which play an important role in water quality management. However, research of aquatic organism criteria for pesticide is still in the early stages in China. On this basis, study on the aquatic organism criteria for Endosulfan in the Yangtze River Delta Region was conducted. Endosulfan is a pesticide which has been widely concerned due to its high toxicity and persistence, it was included in the list of Stockholm convention on POPs in 2011. However, Endosulfan is still in production and use in the Yangtze River Delta Region where is not only the area with most concentrated pesticide production enterprises, but also the area with high rates of pesticide use. In this study, 15 kinds of representative aquatic organisms of Yangtze River Delta Region were selected, among which, there were 11 species of freshwater animals in 11 different families, 2 freshwater algae in 2 different families, and 2 vascular plants in the family Lemnaceae. The toxicity data of Endosulfan to the aquatic organisms was obtained through acute and chronic tests, and then Toxicity Percentile Rank method (the USEPA methodology, 1985) was used to derive the aquatic organism criteria for Endosulfan. The results showed that Final Acute Value was 0.72 μg/L, Final Chronic Value was 0.047 μg/L, and Final Plant Value was 2.74 mg/L. According to Toxicity Percentile Rank method, the acute criterion was equal to one-half the Final Acute Value, and the chronic criterion was equal to the lowest value of the Final Chronic Value and the Final Plant Value. Therefore the acute and chronic criteria for Endosulfan in the Yangtze River Delta Region were 0.36 μg/L and 0.047 μg/L respectively. The results of this study could be used in formulating water quality standard and assessing aquatic risk for Endosulfan.

TU048

Xenopus oocyte responses after cadmium and lead exposures: a cell biology investigation

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Cadmium and lead are recognized as priority contaminants to be monitored in the aquatic environments. They are toxic (behavioural, morphological, cellular and biochemical disturbances) for numerous organisms. Amphibians are especially threatened by contaminant exposures, whatever the stage of their life cycle. Our previous studies demonstrated that Cd, but not Pb, seriously impacted different aspects of the *Xenopus laevis*' oocyte maturation. From these observations, we aim to highlight specific targets of metal contaminant exposures in the xenopus oocyte. Thus, (1) meiosis resumption kinetic studies, (2) analysis of meiotic spindle formation and (3) immuno-detection of some major biochemical actors of the maturation process (Mitogen Activated Protein Kinase pathway, MAPK, and M-phase Promoting Factor, MPF) were conducted. The same concentrations tested in previous studies (0 to 25 mg.L^{-1} of metal chlorides) were used. For Cd, (1) we observed only for 25 mg.L^{-1}, that the germinal vesical breakdown (GVBD) was slowed down and also non hormono-induced maturations. (2) Ectopic and/or abnormal spindles as well as disorganised chromosomes were present for all exposure concentrations (0.25 to 25 mg.L^{-1}). (3) No deregulation in the MAPK pathway was engendered, but disturbances in the MPF pathway were reported. This last result could be related to the cytological observations. Concerning Pb, (1) GVBD kinetics showed contrasted profiles. It seemed that a delay occurred at the end for 0.25 mg.L^{-1}. In contrast, for 25 mg.L^{-1}, a transitory acceleration was recorded during the maturation. No other effect in term of cytological and biochemical analysis (2 and 3) were observed for Pb exposures. From all these results, we can conclude that Cd and Pb did not affect the same cellular targets and could have specific exposure signatures. This study, in addition to our previous ones, contributed to better understand the effects of these metal contaminants in the first stages of the amphibian life cycle: oocyte and embryo. With similar approaches, works with other contaminants are in progress.

TU049
Ecotoxicity - miniaturized tools for microorganisms growth
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The proposed subject aims to develop miniaturized ecotoxicity tests with millifluidic devices. The regulatory context at both European and International level evolves rapidly thus relevant assessment methods are needed for rapid acquisition of possible toxicological determinants for industrial substances. Ecotoxicity tests are a major challenge because they use animal testing to a limited extent and should be limited as much as possible. However, these tests are essential in order to determine the acute and chronic environmental toxicity of substances to different living organisms: microalgae, daphnia, fish (for the aquatic compartment), bacteria, worms, or plants (for the terrestrial compartment). We propose here to develop and adapt some of these tests in miniaturized fluidic systems in order to benefit from the screening capacities of these tools. In particular, we would like to focus on testing with microorganisms which size is compatible with our tools. For these reasons, we have adapted the chronic toxicity tests from microalgae (standard NF EN ISO 8692) in our millifluidic design. We use a millifluidic approach in which the microorganisms are confined within a drop containing the chemical substance. The droplets are transported in a continuous immiscible phase. Drops generated in the millifluidic systems can circulate in the flow channels but also be stored. Since each of the drops represents a replicate, it is possible to generate several drops of identical or variable composition; it becomes possible to access a large amount of information. This makes it possible, for example, to have a high statistical power for a given condition or to screen several compositions in parallel and thus accelerate the data acquisition phases. Preliminary tests have been performed to follow the growth of Pseudokirchneriella subcapitata in microdroplets with various type of tubing. We observe that the growth is higher in PTFE tubing, probably due to a higher oxygen permeability if we compare it to glass tubes. Other tests have been done at various concentrations of a reference substance (3-5 dichlorophenol) and show the inhibition of growth of algae in comparison to the control. Relation between the classical test in Erlenmeyer has also been investigated. We plan to adapt non-intrusive quantitative online growth measurements to follow automatically the Algae growth in several miniaturized droplet tubing to evaluate the toxicity of various products.

TU050
Environmental implications and potential human risk linked to Tributyltin contamination in Northern Chile
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An integrated environmental assessment of imposex and butyltin (BT) contamination was done using surface sediments and tissues of *Thaisella chocolata*(an edible gastropod) from northern Chile. Exceptionally high TBT impacts caused by fresh inputs were observed along the Caldera coastal zones. The BT levels detected in surface sediments classifies nine out of twelve studied sites as highly (109 – 365 ng Sn g^{-1}) or more than highly (> 365 ng Sn g^{-1}) contaminated. In addition, widespread imposex occurrence (11 out of 12 sites) associated to high incidence of sterility indicates that environmental levels were high enough to cause deleterious effects on the local biota. Moreover, imposex and BT concentrations found in “Isla Grande Atacama” (sites S7, S8 and S9) indicates a limited effectiveness of this marine protected area designed to conserve the local biodiversity, as well as, geopolitical and social interests.The spatial distribution of both, butyltin and imposex levels, was related to the profile and intensity of ship/boat traffic. However, areas under influence of small to medium boats (marinas and fishing harbors) presented higher levels of contamination. This observation corroborates to recent South American studies which has pointed out marinas and fishing harbors as current sources of fresh TBT inputs. Beyond that, oceanographic conditions such as coastal currents, upwelling system and the presence of closed bays were also determinant to the distribution of BT residues along the studied area.The ingestion of only about 100 g of “locate” foot tissue (4 to 8 organisms) caught from the most contaminated sites (S1, S3, S4, S5 and S6) may certainly lead to BT intake exceeding the TDI recommended by European Food Safety Authority. Thus, the local population consuming this marine resource from Caldera may be under dietary risk. Moreover, the same areas identified as heavily contaminated are also used for fishing (artisanal and industrial) and farming of other marine resources, which certainly also contribute to increase the daily intake of BT. The environmental and human health implications detected in Caldera confirm TBT contamination as an environmental issue far from being solved within South American coastal areas. Thus, considering the high environment impacts and the potential human health risk associated to seafood consumption, regulatory actions toward environmental protection and food safety of local populations should be implemented.

TU051
Assessment of sperm quality in palaemonid prawns using Comet assay: methodological optimization.
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The aim of this study was to adapt the comet assay in spermatozoa of the marine prawn *Palaemon serratus* to use it as a marker of sperm quality. Indeed, due to the characteristics of their spermatozoa, the measurement of DNA integrity is ones of the few markers, which can be transferred to crustaceans to assess the quality of their semen. In first time, the methods to collect and to maintain spermatozoa were optimized. The cell survival was estimated during kinetics of preservation (i.e. 1, 2, 4 and 8 h) in various suspension media to define the artificial seawater (ASW) as optimal. Several methods in the releasing of spermatozoa from the spermatophore of prawns were estimated with regard to their incidence both on the efficiency of extraction and the survival of cells. The pipetting up and down turned out to be the most successful and the least invasive technic. Secondly, the transfer of Comet assay was optimized by studying various times in both cell lysis (i.e. 1, 6, 18 h) and DNA denaturation (i.e. 15, 30 and 45 min), after *in vitro* exposure of spermatozoa toward a H_2O_2 gradient as model genotoxicant. Results revealed that a minimum of 1 h in cell lysis and 15 min of DNA denaturation was sufficient to obtain valuable results, linked with a low compaction of DNA in spermatozoa of *Palaemon sp*. Finally, the sensitivity of *P. serratus* spermatozoa was assessed after *in vitro* exposures toward model genotoxicants displaying various modes of interaction with DNA (i.e. UV-C, 5-30 sec; H_2O_2, 5-10 μM and MMS, 0.5-5 mM) and some environmental contaminants known or suspected to be genotoxic (i.e. cadmium and diuron, 0.015-1.5 μg.L^{-1}; carbamazepine, 0.1-10 μg.L^{-1}) toward invertebrates. The low variability of the baseline level of DNA strand breaks recorded in controls highlighted the robustness of the method. *P. serratus* spermatozoa displayed significant DNA damage from the lowest doses tested for all model genotoxicants, but conversely, no genotoxic effect of tested environmental contaminants was observed. These results could suggest a difference in the response or sensitivity of spermatozoa to environmental genotoxicity between invertebrate species, and therefore the interest of Palaemonidae prawns in ecogenotoxicology. In conclusion, the present study underlines the potential of the Comet assay as a marker to assess the contamination impact on the sperm quality in Palaemonidae prawns in view to a potential application for *in situ* biomonitoring surveys.

TU052
Acute vs Chronic testing: What does this mean for protecting the marine environment?
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The regulation of chemicals entering the marine environment often focuses on lethal endpoints, short-term exposure and adult life stages, which do not always indicate the true extent of toxicity. Here we look at both acute and chronic testing of *Corophium volutator* and *Tisbe battagliai,* the chronic tests of which include a

number of sub lethal endpoints. The same test chemical was used in all experiments. The acute *C. volutator* test was a 10 day whole sediment bioassay and the acute *T. battagliai* was a 5 day semi static assay. For the chronic tests, as well as mortalities, the weights and lengths of *C. volutator* were recorded after 28 days and the number of broods and number of nauplii produced by *T. battagliai* were recorded after 21 days. Both acute tests showed no significant mortalities in any test concentrations (up to 4750 mg/kg for *C. volutator* and 1000 mg/L for *T. battagliai*) compared to the controls. However, the chronic *C. volutator* test showed a NOEC, LOEC and EC_{50} of 1000, 2200 and 2342 mg/kg respectively for survival. Similarly, the *T. battagliai* chronic test showed a survival EC_{50} of 396.2 mg/L with a NOEC and LOEC of 78 and 224 mg/L respectively. The *C. volutator* length and weight data also showed increased toxicity of the test item compared to the acute test with the lowest NOEC at < 460 mg/kg for length data. Overall the toxicity in the *T. battagliai* chronic test was higher than the acute with EC_{50s} of214 mg/L for number of broods and 274 mg/L for number of nauplii produced. The work conducted here indicates that the acute tests usually accepted for submission to regulatory authorities do not always indicate the true extent of the toxicity of chemicals to marine organisms. Not only were mortalities seen to be higher (with lower EC_{50s}) over the longer duration of the chronic tests, but sub lethal effects were also demonstrated that could have significant implications for population survival and even impact the wider community. This highlights the need to revise the regulatory testing approach and to develop and standardize both acute *and* chronic tests for the evaluation of the impacts of chemicals, in order to protect marine resources.

TU053

Aquatic toxicity of biofuels - acute toxicity, developmental toxicity and reproductive toxicity of four biofuels candidates

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Development and production of biofuels is rising due to the increasing global demand for energy. This implies an increased risk for a release into the environment. Aquatic systems are particularly considered to be vulnerable to fuel contaminations. Ecotoxicological biotests can be applied in a prospective assessment of biofuels and enable an early identification of the aquatic toxicity of biofuels even during their development. This study focuses on the investigation of four biomass-derived fuel candidates: 2-methylfuran (2-MF), 2-butanone (MEK), 2-methyltetrahydrofuran (2-MTHF) and 1-Octanol (1-Oct). These substances are considered very promising alternative fuels for spark-ignition engine ("gasoline engine"; 2-MF, MEK) or compression-ignition engine ("diesel engine"; 2-MTHF, 1-Octanol). *Daphnia magna* and *Danio rerio* were used to investigate the aquatic toxicity of these four biofuel candidates. Acute embryotoxic and developmental effects were investigated in the fish embryo toxicity test with *D. rerio* (OECD 236). Moreover, acute toxicity and reproduction toxicity were investigated in the acute immobilization assay with *D. magna* (OECD 202)and the *D. magna* reproduction test (OECD 211). The toxicity testing revealed a very low acute and developmental toxicity for MEK and 2-MTHF compared to 2-MF and 1-Oct. For MEK and 2-MTHF, EC_{50} >1000 mg/L were found for *D. magna* immobilization and LC_{50} >2000 mg/L (2-MTHF) and >4000 mg/L (MEK) were found for *D. rerio* embryo toxicity. EC_{50}< 35 mg/L were determined for acute immobilization induced by 2-MF and 1-Oct and fish embryo toxicity testing revealed lower LC_{50} for 2-MF (< 500 mg/L) and for 1-Oct (around 15 mg/L). Fish embryos exposed to 1-Oct showed the most significant deformations, such as spinal curvature, in 15 mg/L exposure. Effects on *D. magna* reproduction were found for concentrations of ≥14 mg/L (2-MF) and ≥270 mg/L (2-MTHF). The overall results showed that the rank of acute, developmental and reproductive effects of the biofuel candidates is 1-Oct>2-MF>2-MTHF>MEK. These findings can be used in the further fuel-design process and enable the focus on biofuel candidates with a lower aquatic toxic potency. This work was performed as part of the Research Cluster "Tailor-made fuels from biomass" funded by the Excellence Initiative by the German federal and state governments to promote science and research at German universities.

TU054

Cross-species assay validation using the AOP "deiodinase inhibition leading to impaired posterior chamber inflation"

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The Adverse Outcome Pathway (AOP) concept is increasingly being recognized as a promising conceptual framework for describing toxicity pathways, which contains information that is sufficient to predict an adverse outcome of regulatory importance. Previously, we assessed the feasibility of developing an alternative, mechanistically informative testing strategy to replace the chronic Fish Early-Life Stage test (FELS, OECD TG 210), using an AOP-based approach. We developed an AOP encompassing deiodinase (DIO) inhibition resulting in decreased T3 concentrations leading to impaired swim bladder inflation in fish. *In vitro* assays were used to measure DIO enzyme activity of 51 relevant compounds. Using these results, *in vivo* effects on swim bladder inflation were predicted. These predictions were biologically validated for a set of 14 compounds, with the exception of only 2 false positives and 1 false negative, using zebrafish as model organism. Our results were in line with our AOP and illustrate how AOP-derived information can be used for assay development and refinement. In a next step, we assessed the cross-species applicability of our AOP-based assays. In order to predict an AO based on a molecular initiating event (MIE) or key event, one needs to take into account the fact that the affinity of certain compounds to interact with receptors, enzymes etc. can differ among species. As DIO1 inhibition is the MIE of our AOP on which an *in vitro* assay was based, we investigated whether the use of porcine, rat or fish liver as starting material would result in similar *in vitro* DIO1 inhibition patterns for a set of 22 compounds, and thus whether predictions in one species can be made based on assays using tissues of other species as the starting material. Results show that the DIO1 inhibitory potential compared to a reference compound is nearly identical between the three selected species. However, a set of bisphenol A derivatives showed lower inhibition potential in fish and rat compared to pig. A reassessment of the validation experiments based on zebrafish inhibition data shows that TCBPA is no longer a false positive prediction, while BPA appears to become a false negative prediction. These results show that for most compounds, tissue originating from different species can be used in our DIO1 assay to predict apical outcomes in fish. Furthermore, it demonstrates that AOPs can support cross-species extrapolation after investigating their taxonomic applicability.

TU055

Comparing the acute sensitivity of growth and photosynthetic endpoints in three Lemna species exposed to four herbicides

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An ecological impact of four herbicides (atrazine, diuron, paraquat and simazine) was assessed using the aquatic floating vascular plants, *Lemna gibba*, *Lemna minor* and *Lemna paucicostata* as test organisms. The sensitivity of several ecologically relevant parameters (increase in frond area, root length after regrowth, maximum and effective quantum yield of PSII and maximum electron transport rate (ETR_{max}), were compared after a 72 h exposure to herbicides. The present test methods require relatively small sample volume (3 mL), shorter exposure times (72 h), simple and quick analytical procedures as compared with standard *Lemna* assays. Sensitivity ranking of endpoints, based on EC_{50} values, differed depending on the herbicide. The most toxic herbicides were diuron and paraquat and the most sensitive endpoints were root length (6.0 – 12.3 μg L^{-1}) and ETR_{max} (4.7 – 10.3 μg L^{-1}) for paraquat and effective quantum yield (6.8 – 10.4 μg L^{-1}) for diuron. Growth and chlorophyll *a* fluorescence parameters in all three *Lemna* species were sensitive enough to detect toxic levels of diuron and paraquat in water samples in excess of allowable concentrations set by international standards. CV values of all EC_{50}s obtained from the *Lemna* tests were in the range of 2.8 – 24.33%, indicating a high level of repeatability comparable to the desirable level of < 30% for adoption of toxicity test methods as international standards. Our new *Lemna* methods may provide useful information for the assessment of toxicity risk of residual herbicides in aquatic ecosystems.

TU056

Inhibitory Effects of Ammonia on Filtering of Commensal and Pathogenic Escherichia coli by the Cladoceran Daphnia magna

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Enteric bacteria such as *Escherichia coli* frequently occur as contaminants in surface waters. Interestingly, grazing by cladocerans can reduce the abundance of many enteric bacteria associated with fecal pollution. In this study, we investigated the potential of *Daphnia magna* to filter commensal and pathogenic *Escherichia coli*, and we examined the inhibitory effects of bile acids and unionized ammonia. Grazing on commensal and pathogenic *E. coli* of human origin was comparable, and slightly greater at 20 compared to 15 and 25 °C. Filtering activity was strongly dependent on *D. magna* and *E. coli* densities at environmentally relevant bacterialconcentrations. Maximum feeding rates were >10^7 bacteria h^{-1} daphnid^{-1}. Clearance rates were 1-6 mL h^{-1} daphnid^{-1}, and filtering was independent of bacterial cell sizes between 0.7 and 1.8 μm. However, filtering and ingestion of *E. coli* by *D. magna* was susceptible to acute inhibition by unionized ammonia with a 24 h EC50 of 0.18 mg L^{-1} NH_3-N. The lowest observed effect concentration (LOEC) for unionized ammonia was 0.09 mg L^{-1} NH_3-N. In contrast, filtering of *E. coli* by *D. magna* was not inhibited by bile acids at concentrations up to 125 mg L^{-1}. The results indicate that ammonia toxicity should be considered when applying

Daphnia for attenuation of fecal pollution in e.g., tertiary treated wastewater and constructed wetlands. However, optimizing conditions for *Daphnia* can facilitate pollution free biological disinfection of enteric pathogens (zooremediation).

TU057

Levels of POPS in Marine Farmed and Wild Fish From Tanzania, A Pilot Study

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Aquaculture is a fast growing industry in Tanzania. Farming of milkfish (*Chanos chanos*) and mullet (*Mugil cephalus*) have become an important activity for food source and income generation to the Tanzanian coastal population. However, no systematic study has been done to assess the quality and health of marine farmed fish in connection to harmful chemical. To investigate the level and occurrence of harmful chemicals, farmed and wild milkfish and mullet from the coastal area in southern Tanzania (Mtwara) and Zanzibar islands were collected and analysed for persistent organic pollutants (POPs). Where possible, fish of about the same size were collected in every location in order to obtain comparable information. Preliminary results show that *p,p'*-DDE is the major pollutant in livers of most fishes. The highest *p,p'*-DDE levels were found in wild milkfish from Mtwara ranging from < LOD-622 ng/g lipid weight (lw). This maximum level was 400 times higher than the highest level found in farmed milkfish from ponds adjacent to sea (1.5 ng/g lw), suggesting that the fish in Mtwara pond was exposed to very low *p,p'*-DDE levels. *p,p'*-DDT was detected in milkfish from Jozani and Shakani ponds in levels of 14 and 3.5 ng/g lw respectively, while 4 ng/g lw in wild mullet from Pemba. Levels of ∑10PCB (PCB 28, 52, 74, 99, 118, 153, 138, 180, 170) were low and ranging between < LOD-5.56 ng/g lw. The highest levels of ∑10PCBs were found in milkfish from Shakani pond at Unguja (5.65 ng/g lw). The PCB pattern was dominated by PCB 153>PCB 180>138. Levels of HCB, HCHs, and trans-nonachlor were low and may reflect background levels. PBDEs were detected in low and varying levels in all locations. BDE 47 was the dominating congener, and was found in highest levels (0.13 ng/g lw) in the fish from Shakani at Unguja. HBCD was only detected in farmed milkfish from Unguja in levels ranging between 0.09-0.16 ng/g lw. POP levels in mullet were in the same range as milkfish. Compared to lipid based levels detected in muscle of wild tilapia from the big lakes in Tanzania, the present POP levels in liver are much lower, except for *p,p'*-DDE in the wild milkfish from Mtwara and wild Mullet from Pemba, which are in the same range as levels found in Lake Tanganyika. The measured POPs levels in the studied fish were all below MRLs of EU and the fish are considered safe for human consumption however, they may pose a risk to the fish species and threaten biodiversity

TU058

Setting up aquatic toxicity testing using zebrafish, daphnia and algae

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Aquatic toxicity testing is stipulated for environmental hazard and risk assessment by the regulatory framework on chemicals (REACH), plant protection products, biocides, pharmaceuticals and feed additives. Biobide is a biotechnology company offering toxicity and efficacy services to Pharma, Biotech, Chemical, Cosmetic and Nutraceutical companies with zebrafish. Based on this experience is setting up guidelines for aquatic toxicity testing of chemicals under Good laboratory practices (GLPs) as the aquatic toxicity package. As requirements of different international regulatory organism varies slightly, we harmonize guidelines to offer services that fit the most organisms. Aquatic toxicity refers to the effects of a compound to organisms living in the water and is usually determined on organisms representing the three trophic levels: plants (algae), invertebrates (crustaceans as Daphnia spp.), and vertebrates (fish). Alga growth inhibition test determines the effect of a substance on the growth of freshwater algae (E_rC_{50}); Daphnia acute immobilization test determines the concentrations which 50% of the daphnia are immobilized (EC_{50}); and Acute fish toxicity determines the concentration which is lethal to 50% of the fish (LC_{50}). In this work the presence of the issues found will be discussed.

TU059

Vertically-transmitted microsporidia parasite could disturb the endocrine disruption assessment in Gammarus sp.

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Endocrine disruptors (EDCs) are well known to disrupt the development and the reproduction of exposed organisms. Although this point has been studied in vertebrate models, the limited knowledge of the endocrine system of invertebrates makes the evaluation of EDCs effects difficult. However, invertebrates represent the major part of aquatic ecosystems, such as amphipods *Gammaridea*, which are crucial for their functioning (e.g. litter degradation, food resource). Nevertheless, gammarids are hosts of hidden parasites such as vertically-transmitted microsporidia (microsporidia VT), which could be confounding factors in assessment of EDC effects. Indeed, some microsporidia VT could have endocrine effects by their own present in the host, since it was observed, for example, a feminisation of juvenile males in some *Gammarus* sp. The present study aimed to assess the effects of the ethinylestradiol (EE_2) and the cyproterone acetate (CPA), EDCs commonly studied in vertebrates, and the presence of microsporidia VT, on the spermatozoa production of the freshwater amphipod *Gammarus pulex* males, by assessing the number of spermatozoa produced as well as the their length. Results in control conditions (i.e. unexposed males) revealed that the presence of *Dictyocoela duebenum* (the only one microsporidia VT in the studied *Gammarus pulex* population) significantly reduces the production of spermatozoa compared to unparasitized males, without having an effect on their length. In addition, it appeared the EE_2 did not affected nether the number of spermatozoa produced, nor their length, while CPA stress caused a decrease of the spermatozoa production in parasitized *G. pulex* males compared to respective controls. This work highlighted EDCs affecting vertebrates could also impact invertebrates species. In addition, the presence of microsporidia VT appeared to be a confounding factor which could led to misinterpretation the endocrine risk assessment. Finally, results supposed that both EDCs and parasites interact with the androgenic gland, but further studies are needed to understand their mechanisms of action.

TU060

STUDIES OF TEMPORAL VARIATIONS OF FISH BIOMARKER EXPRESSION IN A SWEDISH COASTAL SITE

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The health status of two fish species, perch (Perca fluviatilis) and eelpout (Zoarces viviparus), has been studied yearly in a program for integrated coastal fish monitoring in four costal reference sites in Sweden since 1988. The selected reference sites are characterised by no or small local sources of contaminants. The fish health is assessed to provide indications of impacts of pollutants or other stressors in the coastal environment. Together with measurements of contaminants and fish stock assessment, the fish health studies form an integrated fish monitoring program. With a set of physiological and biochemical endpoints (i.e. biomarkers) all of the sites show "early warning" signs for impaired health status for the two species. For example in female perch from the Baltic Sea coastal sites, a 20-30% reduction of gonad size and fewer eggs, and marked up to fivefold increase of the EROD activity has been observed. Other biomarker time trends in perch and eelpout include increases in plasma calcium content, blood glucose level and in lymphocyte number and oxidative stress. It has not been possible to explain these time trends in the physiological and biochemical biomarkers by changes in exposure to the contaminants that are routinely monitored. In addition to that the biomarker data from the perch in costal sites in the Baltic Sea show clear time trends some of the biomarkers show large variations between years. To further investigate and compare between years variations in the perch biomarkers two years, 2010 and 2014, showing large variations were selected for further studies. The biomarker data from these two years showed for example that the EROD activities, plasma levels of vitellogenin and gonad sizes were markedly higher in 2010 than in 2014 in the female perch, whereas catalase and glutathione reductase activities and plasma content of calcium were markedly lower in 2010 than in 2014. To provide more information of possible differences stored samples of perch liver from the 2010 and 2014 sampling were selected for RNA sequencing. The purpose was to identify differences in gene transcription patterns that might explain observed differences between years that might be caused by different exposure scenarios. In addition, the RNAseq approach provided a sequenced perch transcriptome that can be used in future studies.

TU061

Effects of imidacloprid on the escape and agonistic behaviors of crayfish

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Following the release of imidacloprid in 1991 by Bayer CropScience, nicotinoid insecticides became the fastest growing class of chemical insecticides on the global market. However, concern has grown over the potential harm that imidacloprid and other nicotinoids pose to non-target organisms, especially through sublethal effects on reproduction, behavior, physiology and development. In insects, imidacloprid binds to nicotinic acetylcholine receptors (nAChR) and interrupts synaptic transmission leading to a quick death. Imidacloprid readily dissolves in water and can have effects on non-target aquatic organisms such as crayfish. Crayfish play important ecological roles in freshwater ecosystems, where they facilitate leaf litter breakdown, modify benthic sediments through bioturbation and function as both predators and prey. For this reason, the sublethal effects of imidacloprid on crayfish behavior could have ecosystem-wide impacts. This study investigated several behaviors in the rusty crayfish *O. rusticus* that would be impacted if sublethal doses of imidacloprid impacted nerve functions, including escape (tailflipping), aggression (claw raising and pinching), and righting ability. Crayfish (7 per group) were exposed individually for 0, 1, 10 and 100 μg/L of imidacloprid for 10 days and imidacloprid doses were refreshed every second day during water change. Escape and agonistic behavior was examined with the poke test. The poke assay consisted of a one minute acclimation period, followed by a poke with a glass rod into the crayfish's container at a 90 degree angle from the bottom and about 0.5 cm directly in front of the crayfish. Responses were scored on a scale of 1-5, from least to most aggressive; 1 = tail flip, 2 = back away, 3 = no response (neutral), 4 = lift and spread claws and/or move forward, 5 = grab the rod. Results show that crayfish from all exposure groups became more passive with an increased frequency of neutral responses to the stimulus. In the100 μg/L group an increase in the frequency of neutral responses was detected already after 4 days exposure and after 8 days in the low (1μg/L) exposure group. Several studies have reported levels of imidacloprid exceeding this study's lowest exposure scenario, 1 µg/l, and we therefore conclude that environmental relevant levels of imidacloprid will have negative effect in crayfish, possibly leading to negative effects in freshwater ecosystems.

TU062

Environmental risk assessment of ionic liquid [omim][BF4]: the role of a chaotropic agent (Gnd HCl) in understanding ILs' mode of action

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Ionic liquids (ILs) are chemicals with unique properties and considered as ideal environmental friendly substitutes of conventional solvents. Among them, 1-octyl-3-methylimidazolium tetrafluoroborate ([omim][BF_4]), widely used either pure or in mixtures with water and/or conventional solvents such as acetone, might pose a significant environmental risk, which significantly depends on its structure and mixture with water or carrier solvents. In this context, the present study investigated the adverse effects of [omim][BF_4]*on mussel hemocytes*, as well as whether acetone could mediate their toxic potency. In addition, since understanding ILs' toxic mode of action, with and/or without the presence of conventional solvents, toward non-target species is of great concern for their development, application and safety, guanidine hydrochloride (Gnd HCl), a chaotropic agent and strong macromolecules' denature, was used for elucidating the hydrogen bonding network among [omim][BF_4]/water and/or [omim][BF_4]/water/acetone with exposed cells and shed light to [omim][BF_4] mode of action. Specifically, cytotoxicity (with the use of neutral red retention assay/NRRT), oxidative stress (in terms of superoxide anion and lipid peroxidation byproduct (malondialdehyde/MDA) formation) and DNA damage (with the use of Comet Assay), with and/or without the presence of 0.06% v/v acetone were studied. Thereafter, all assays were repeated with pre-treatment of cells with Gnd HCl (1 mM) for 15 min before exposure to the sub-lethal concentrations of the toxic agent. According to the results, concentrations of [omim][BF_4] ranging from 0.2 to 1 mg L^{-1} were able to induce cytotoxic (almost < 50% reduction of cell viability), oxidative (increased levels of $^{\cdot}O_2^-$ production and lipid peroxidation by-products) and genotoxic (increased levels of DNA damage) effects on mussel hemocytes, while cells treated either with acetone or pre-treated with Gnd HCl 1mM showed a significant diminishment of [omim][BF_4]-mediated toxic potency in all cases. To our knowledge, the current study showed for the first time that chaotropic agents, such as Gnd HCl, could contribute to the understanding of ILs-mediated toxic mode of action, thus serving as an important agent in our effort to elucidate [omim][BF_4] structural modifications in the presence of acetone.

TU063

An experimental approach to improve the understanding of trophic transfer of selenium in freshwater environments and its potential hazard in fish

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Selenium (Se) is a naturally occurring essential element but also a pollutant of great concern in North America and elsewhere. Anthropogenic activities such as mining operations represent significant sources for Se into aquatic environments where it is usually found in inorganic forms (e.g., selenite and selenate) in the water phase. These forms of Se are rapidly biotransformed to organic species of Se (e.g. selenomethionine; SeMet) by algae and microorganisms, thereby increasing the bioavailability of Se for higher trophic taxa such as fish, which can accumulate potentially toxic levels of Se. Today, the risk assessment of selenium is hampered by our incomplete understanding of trophic transfer of Se, especially at the base of the foodweb. Knowledge regarding the processes that govern transformation, assimilation and accumulation of Se as well as the transfer along aquatic food chains would facilitate its regulation.\nTo address this issue, a representative aquatic food chain will be investigated to gain insight into biotransformation, bioaccumulation, transfer along the food chain and toxicity to fish at the upper trophic level. Natural biofilms collected from uncontaminated lakes in Canada were characterized regarding their community composition and exposed to environmentally relevant aquatic concentrations of either selenite or selenate (5 and 25 μg/L each). After 7 d amphipods (*Hyalella azteca*) were allowed to graze on the exposed biofilms, followed by analysis of the Se concentration in the amphipods to determine the tropic transfer and bioaccumulation of Se. Furthermore, the uptake and biotransformation of Se in the biofilm were investigated by analyzing subsamples during the entire study. Ongoing studies will feed *H. azteca* that have accumulated Se to fathead minnows (*Pimephales promelas*) in a reproduction assay, thereby completing the food chain. This will allow to further characterize the transfer of Se in the food chain. In addition, the effects of Se on sexually mature adult fish exposed via the food chain and on the offspring exposed via maternal transfer will be examined. These results will be compared to effects on the development of *P. promelas* embryos injected with SeMet to gain insights into early life stage sensitivity and mechanisms of Se toxicity.\nThe obtained data set can be used to assist in the development of an advanced environmental risk assessment for Se, possibly enabling for site-specific hazards to be better characterized.

TU064

Effects of the organic-UV filter, oxybenzone, on photobiology of the hexacoral Zoanthus sp.

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Coral reefs are extremely complex and productive ecosystems, providing habitats for thousands of marine species. Unfortunately, anthropogenic activities such as the presence of xenobiotics resulted in the degradation of this priceless ecosystem over the past few decades. It is estimated that approximately 40% of coral reefs located along coastal areas are at risk of exposure to organic UV-filters such as oxybenzone (Benzophenone-3; BP-3) widely used in sunscreen lotions and personal-care products. It is important to evaluate the effects of these emerging contaminants on local species inhabiting the more affected areas, namely the intertidal environments of tropical regions. The photosynthetic, hexacoral, *Zoanthus* spp. is an abundant species in coastal areas. These marine invertebrates, that manage to live in physiologically challenging environment where few species survive, have a high potential application as an indicator organism. However the existing research in zoantharian ecology is scarce in comparison with other cnidarian groups. In this study we aimed to evaluate the effect of short exposures of BP-3 on *Zoanthus* sp. photochemical performance and zooxanthellae density. Using a non-invasive method and replicated mini-colonies of 4 to 6 polips**,** the maximum quantum yield of photosystem II (F_v/F_m) was evaluated *in vivo* at the beginning and at the end of the exposure,with a Pulse Amplitude Modulation fluorometer (PAM). After exposure colonies were also sampled to determine the zooxanthellae density. Cell concentration was normalized to *Zoanthus* sp. dry weight. Preliminary results show that exposure to sub-lethal concentrations of BP-3 impairs both photosynthetic efficiency and zooxanthellae density in comparison to control treatments. The results will be discussed taking into account the ecological relevance of the coral species selected, the sensitivity and reliability of both approaches as early warning indicators of bleaching and the potential ecological effects of organic UV-filters in costal ecosystems. **Key-words:** corals; emerging contaminants UV filters; Benzophenone-3; marine ecotoxicology

TU065

Changes of sinking rate and physiochemical composition of two marine diatoms exposed to diuron

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In marine environment,adverse effect of pollutants on physiological status of marine organisms have been considered, however there are no studies on the effects of pollutants particularly antifouling agents on sinking rate of algae including diatoms.Among the antifouling agents diuron also known as DCMU,have been used all over the world.Diuron has been detected in coastal areas of many countries. More over sinking rate control is important for phytoplankton population specially for diatoms as they are responsible for oceanic carbon and silicon flux.The aim of this work is to investigate the effect of diuron on sinking rate and the physiochemical changes that regulate the sinking rate of two marine diatoms ,*Thalassiosira pseudonana* (single-celled species) and *Skeletonema marinoi-dohrnii* complex (chain forming species).The sinking rate of both diatoms exposed to diuron at a level of 50% effective concentration for growth (EC50) decreased significantly compared with control at 72h.Photosynthetic performance (Fv/Fm and PI_{ABS}) of both diatoms also decreased significantly with diuron exposure.The number of cells per chain in *S. marinoi-dohrnii* decreased significantly with diuron treatment but *T. pseudonana* cell diameter remained stable.Neutral lipid content per cell was significantly higher compared with control at 72h in both diatoms in the EC50 level diuron.And water soluble protein concentration per cell was lower than control at 72h in the *T. pseudonana* EC50 group only.Thus our present study suggested that suppression of photosynthetic performance and the resultant physiochemical changes induced the decreased sinking rate that may inhibit the normal survival strategy of diatoms through photoinhibition or delay resting spore formation.More over in the present study,MEC:NOEC>1,that indicated the possible environmental risk of diuron.Therefor the use of antifouling agents should be considered for the ecotoxicological risk assessment of marine primary productivity.

TU066

LABORATORY ASSAYS WITH NON-FORCED EXPOSURE TO PREDICT THE PREFERENTIAL SPATIAL DISTRIBUTION OF FISH IN TWO ECUADORIAN RIVERS

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Laboratory aquatic ecotoxicity assays used to provide data for ecological risk assessments assume that, under natural conditions, organisms living in a contaminated habitat are mandatorily and continuously exposed to contaminants. This assumption neglects the ability of the organisms to detect and avoid contamination moving towards less disturbed habitats such as expected in fluvial systems. Along a river, contaminants can be dispersed forming a gradient or even be patchy distributed, conditioning the habitat selection process by organisms as well as their avoidance and preference behavior. Therefore, in the present study, we assessed the avoidance and preference responses of the model fish *Danio rerio* when exposed to water samples from two Ecuadorian rivers (Pescadillo River and Oro River) with different disturbance levels. A non-forced exposure system, in which water samples from different river points are simultaneously assayed, allowing organisms to move freely between river samples and select the most favorable sample, was used. Results showed that organisms presented a trend to avoid Pescadillo River upstream samples, moving downstream towards to the confluence zone with Oro River. On the other hand, fish exposed to Oro River samples preferred moving upstream. When exposed to samples from both rivers simultaneously, fish tended to prefer Oro River samples. These results leaded us to predict that, as both rivers are connected, fish avoiding environmental disturbers in Pescadillo River would move to Oro River. Therefore, effects of potential stressful conditions present in Pescadillo River that trigger avoidance response by fish may depress fish populations in that river and, indirectly, affect Oro River by inducing an unexpected fish immigration.

TU067

Metatranscriptomic profiles of marine microbial communities responding to complex mixtures of organic pollutants in Arctic and Antarctic waters

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Organic pollutants (OPs), including persistent OPs (POPs), are ubiquitous in the environment and constitute one of the vector of global change due to their ability of modify the composition of the biosphere. However, their impact on marine ecosystems is still largely unexplored. Analyzing their impact on pristine microbial communities, such as polar waters, can provide the baseline of responses in the natural environment. OPs reach polar seawaters mainly through atmospheric deposition. The working hypothesis of this work was that the complex mixture of OPs present in remote polar seawaters can affect both the functionality and the diversity of polar communities and can be tracked by means of metatranscriptomic approaches. We challenged natural polar communities with mixtures of OPs generated by concentrating seawater from the Arctic and Southern Ocean using XAD-2 amberlites. The non-polar fractions of these extracts (referred to as complex mixtures or CMs) were used as experimental treatments to simulate an enrichment of the naturally occurring OPs present in the environment. They include legacy and emerging POPs, aliphatic and aromatic hydrocarbons, and a large fraction of unresolved organic compounds, as determined by standard chemical analyses. Two liters of seawater from two pristine environments, Ny Alesund (Svalbard Islands, Arctic) and Livingston Island (South Shetlands, Antarctica), were incubated for 30 min and 24 h with either local seawater or with seawater fortified with 10-fold local CM concentrations, calculated from their OP content. Treatment effect on growth rates was monitored by flow cytometry. Collected DNA and RNA were processed and sequenced using HiSeq 2000 Illumina. Significant differences were observed between control and treatments in energy and carbohydrate metabolism gene categories, particularly between phylogenetic groups, indicating that responses were taxon-specific. As a general trend, metabolic pathways linked to high growth rates decreased in bacteria with low DNA-content (such as SAR11) and increased in bacteria with high DNA-content (such as Roseobacteria). This work will elucidate further effects of the myriad of OPs present in seawater at trace levels on microbial structure and functions.

TU068

Microbial responses to diffusive pollution in seawater

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The ecological impact and toxicity of seawater organic pollutants (OPs) seem related not to the presence of a single compound but to their myriad co-occurrence. Toxic effect of mixtures of OPs have been observed on phytoplankton cells, with higher lethal effects on the smaller cells. However, effects on heterotrophic microbial communities, the main respirers and recyclers of the large pool of dissolved organic matter, remain uncharacterized. The objetive of this work was analyzing the effects of complex mixtures of OPs found in seawater at environmental relevant levels on the metabolic capacity of microbial communities by measuring gene expression profiles of natural oceanic communities (metatranscriptomics) from Pacific and Atlantic waters. The groups showing the strongest response to OPs belonged to Proteobacteria, specially Gammaproteobacteria, and the maximal response was observed after 2h of exposure. At the end of the experiment, community composition diverged between enriched and control treatments, indicating an effect of OPs. In general, OPs notably reduced RNA metabolism, although several heterotrophic metabolisms were slightly stimulated, suggesting both positive and (mostly) negative effects of OPs on microbial communities.

TU069

EVALUATION OF WATER QUALITY IN RIVERS ON THE SANTA CATARINA ISLAND, BRAZIL, USING BENTONIC ORGANISMS

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Disorganized urban growth and lack of planning in the urban management of the Island of Santa Catarina (Florianópolis, SC, Brazil) have had disastrous consequences on their ecosystems as is occurring in the Itacorubi River Basin. The objective of this work was to monitor the water quality of this water resource, using the analysis of physico-chemical and biological parameters - benthic fauna - to identify possible environmental impacts. For this purpose, water and sediment samples from three sites were analyzed in the Sertão and Itacorubi rivers, which are interconnected and are part of the Itacorubi Hydrographic Basin. The three collection sites are located in urbanized areas, Morro da Lagoa (ML) exhibits the most preserved margins and Córrego Grande (CG) and São Jorge Park (PSJ) the most impacted by anthropic action. The results obtained with the physicochemical parameters analyzed showed high values for ammonia and for phosphorus and a significant change in the number of fecal coliforms in the three sites, in order of increasing ML Hirudinaeand *Chironomidae*, being that in Córrego Grande the number of individuals much greater than in Morro da Lagoa. In the PSJ site, the most abundant family was *Oligochaeta*. The identification of the genera and species of the organism is in progress. The presence of these families in the water bodies indicates that there is contamination from anthropic origin, corroborating with results found using the physical-chemical analyzes. Chromatographic analyzes by GC / MS are underway to identify the presence of possible pollutants, especially drugs and sterols, in water and sediment samples.

TU070

EFFECTS OF URBANIZATION IN THE ESTUARINE REGION OF RIO BIGUAÇU, SC, BRAZIL

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The discharge of domestic sewage, the disordered occupation of the soil and the consequent destruction of the riparian forest are processes that contribute significantly to the deterioration of the water quality of the water sources. The metropolitan region of Florianopolis has undergone an intense urbanization process in recent years, which has modified the landscape and the quality of life in this region. The objective of the present study was to evaluate the water quality of the Biguaçu River in its estuarine region, in selected sites in order to reflect the anthropic changes occurring in the surroundings. Four sites were chosen, in which sediments and water samples were collected. The sediments were analyzed for the presence of sterols by GC / TOF-MS after extraction with methyl tert-butyl ether. The water samples were analyzed with respect to the parameters: ammonia concentration, total phosphate, total phenols, fecal coliforms and sulfide, according to the methodologies described in Standard Methods (APHA). Concentration of metals - Pb, Cd, Al, Hg and Cr were analyzed by ICP / MS. GC / TOF-MS chromatographic analyzes of sediments and water samples were also performed on extracts obtained using SPE (Strata-X)/dichloromethane. The results obtained showed high concentrations of ammonia and total phosphate, besides high fecal coliforms. Lead was the metal found in concentrations above Brazilian legislation. Between the analyzed sterols, cholesterol and derivatives such as coprostanol were identified at varying concentrations in the sediments of the several sites. Estradiol derivatives and drugs such as ibuprofen were prominent in GC / TOF-MS chromatographic analyzes. The results confirmed the high contamination of the waters of the Biguaçu River by the discharge of domestic sewage.

Mechanistic ecotoxicology of engineered nanomaterials:

TU071
Evaluating and predicting the respiratory hazard of particles used in Li-ion batteries for a safe and sustainable development
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Rechargeable Li-ion batteries (LIB) power most of portable electronics and are under continuous improvement to replace other types of batteries for a more effective energy storage. In view of their increasing production, assessing the possible health impacts of materials contained in LIB appears essential. LIB contain micrometric and relatively insoluble particles, consisting of toxicologically relevant elements. Toxicological data is almost inexistent to properly assess this emerging concern. Here, we evaluated the lung toxicity of 3 leading LIB particles ($LiFePO_4$ or LFP, $Li_4Ti_5O_{12}$ or LTO, and $LiCoO_2$ or LCO) and investigated their mechanisms of action in order to develop an *in vitro* predictive tool of LIB particle lung toxicity. Characterization of LIB particles showed that samples comprised a respirable fraction and had different solubilisation rates at neutral and acidic pH. C57BL/6 mice were exposed to LFP, LTO and LCO (0.5 or 2 mg/mouse by oro-pharyngeal aspiration). Different inflammatory lung responses, including the secretion of pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α), were observed 18 h and 3 d after exposure, suggesting different mechanisms of action. Persistent inflammation was observed 2 m after exposure to LFP or LCO and fibrosis only in LCO lungs. A persistent fraction of LIB particle elements was detected in mouse lungs 2 m after exposure by scanning electron microscopy/energy dispersive X-ray spectrometry (SEM/EDX). Co co-localized with endogenous Fe in LCO lungs, indicating the formation of ferruginous bodies similar to those observed after asbestos fibers exposure. Because of the key role of IL-1β in particle-induced inflammation, we also investigated the lung toxicity of LIB particles in IL-1β deficient mice and showed that LFP- and LCO- induced inflammation was IL-1β dependent while the development of fibrosis in LCO lungs was IL-1β independent. Based on these results, we are currently developing an *in vitro* test based on IL-1β to predict LIB particle lung toxicity. We report for the first time the differential lung toxicity of inhaled LIB particles and, therefore, provide scientific information to address this emerging public health issue. IL-1β plays a role in LFP and LCO-induced inflammation, indicating that an IL-1β *in vitro* test might serve as a predictive tool for evaluating the toxicity of next generation LIB and contribute to a safer-by-design development of LIB.

TU072
Interaction of gold nanoparticles and nickel(II) sulfate affects dendritic cell maturation
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The medical application of gold nanoparticles (GNPs) is promising due to their high biocompatibility, but little is known about their health risk in mixtures with other chemical compounds against a complex biological background. We aimed to study the safety profile of GNPs when combined with nickel, a widely distributed heavy metal that is able to form alloys with gold and has allergenic potential. We focused on allergic sensitization by evaluating *in vitro* maturation of dendritic cells. The physico-chemical interactions between 50-nm GNPs and nickel(II)sulphate in biological matrix were investigated using nanoparticle tracking analysis, centrifugal particle sedimentation, UV-Visible spectroscopy, ICP-MS, ζ-potential determination, and proteomics. While both GNPs and nickel(II) induced a maturation response, the cell activation pattern of their mixture was similar to this of nickel(II), suggesting a competitive interaction. Characterization data indicated that nickel(II) ions did not adsorb onto the GNP surface, but causeda clear shift in the GNP-adhered protein corona composition in biological medium. ICP-MS analyses demonstrated a significant decrease in GNP uptake by the cells in the presence of nickel(II). This study highlights the necessity of assessing nanomaterials' health risks in a complex environment reflecting the real world, and the need to complement such studies with in-depth physico-chemical characterization.

TU073
Human absorption of silver ENM: through particles or ions?
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One of the many questions in the human risk assessment of metallic engineered nanomaterials (ENM) such as silver ENM is whether these ENM dissolve in the gastrointestinal (GI) system or are absorbed and translocated to organs and tissues as intact nanoparticles. It is difficult to elucidate this question as *in situ* measurement of silver ENM is still hampered by technical difficulties, but also because silver nanoparticles can be formed *in vivo* after exposure to silver salts. To tackle these difficulties, we compared the tissue distribution of orally administered silver ENM with the tissue distribution pattern of silver ENM and a silver salt that were administered by intravenous (IV) injection. Female rats were exposed to a single dose of NM-300K, consisting of silver nanoparticles with a mean diameter3. The rats were treated either by oral gavage or by IV injection. During a 24-hour period, urine and feces were collected. After 24 hours, selected tissues were sampled and total Ag concentrations were measured by ICP-MS. After treatment by IV injection with the silver salt, largest Ag concentrations were found in the liver, spleen and pancreas, and Ag was largely excreted (32% of administered dose) via urine and feces. After IV injection with NM-300K, largest Ag concentrations were found in the spleen, and < 1% of the administered dose was excreted via urine and feces. This demonstrates that NM-300K, once present systemically, is circulated as particles and solubilization is limited. After treatment of the animals by oral gavage there was a low absorption of Ag, the absorption after treatment with NM-300K being 10-fold lower than after treatment with AgNO3. As there is a certain amount of soluble Ag present in the administered NM-300K dispersion (~3%), the tissue concentrations were normalized for the soluble Ag dose. After this normalization, the Ag concentrations in tissues after exposure to NM-300K were up to 17 times larger than after exposure to $AgNO_3$. This might indicate that either an additional fraction of NM-300K has been solubilized in the GI tract, or that NM-300K is partially taken up as intact nanoparticles. The Ag distribution pattern in the tissues after treatment with NM-300K resembled, however, more that of ionic Ag after IV injection than that of NM-300K after IV injection. This suggests that it is more likely that NM-300K is partially dissolved in the GI tract and subsequently absorbed and excreted via urine and feces.

TU074
In vitro and in vivo uptake, fate and effects of titanium dioxide nanoparticles (TiO2 NPs) and 3,3',4,4'-tetrachlorobiphenyl (PCB77) in rainbow trout
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TiO_2 NPs are among the most produced and used nanomaterials. Their release into European surface waters can result in exposure of organisms including fish. In the last years enormous efforts have been undertaken to determine their *innate* ecotoxic potential, but their interaction and combined toxicological effects with environmental co-contaminants remains hitherto largely unaddressed. The objective of this study was to investigate how TiO_2 NPs and PCB77 influence each other's uptake, fate and toxicity at different levels of biological organization in the model organism rainbow trout (*Oncorhynchus mykiss*). The uptake, fate and effects of TiO_2 NPs and PCB77 at the cellular level was investigated by exposing cultures of the rainbow trout liver cell line RTL-W1 to the substances both alone and together. Transmission electron microscopy analysis demonstrated that TiO_2 NP were taken up via chlatrin-independent endocytosis and routed to multilamellar vesicles. Intracellular TiO_2 NP accumulation was concomitant with enhanced transcription of antioxidant response genes (NRf2, GCL, GST, GPx, SOD) as measured via RT-qPCR, but did not lead to decrease in cell viability as measured by the Alamarblue and CFDA-AM assay (exposure concentration/time: 1-100 μg/ml / 24 h) suggesting that cells were able to successfully countervail TiO_2 NP-induced oxidative stress. Yet, high intracellular burden of TiO_2 NP affected the cells' response to subsequent PCB77 exposure (0.1, 1 and 10 μM; 24 h). Thus, NP-containing cells showed higher cytochrome P450 1A (CYP1A) expression levels and alterations in their antioxidant response.In order to investigate if combination effects of TiO_2 NP and PCB77 occur as well at the organism level juvenile rainbow trout were exposed via the diet to TiO_2 NP (zero, 1, 10 and 100 μg per g fish per day), PCB77 (10 ng per g fish per day) and mixtures thereof for 14 days. Gill, liver, intestine and brain samples were analysed with respect to changes in histology, gene expression and biochemical parameters. In parallel TiO_2 NP and PCB77 levels were quantified using inductively coupled plasma mass spectrometry and gas chromatography-mass spectrometry, respectively. Preliminary results suggest that toxicokinetic and dynamic combination effects also occur *in vivo*. Our results show that the combined use of *in vitro* studies and *in vivo* studies can help to advance our understanding of mechanisms underlying NP and NP-chemical mixture toxicity.

TU075
Evaluation of the toxic, cytotoxic and genotoxic activity of three composite nanomaterials (CLAY/CNTs, CLAY/ADMA, GO/ADMA)
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Nanocomposites are a new generation of novel materials, which are formed by mixing one or more dissimilar materials at the nanoscale so as to control and develop new and improved structures as well as properties. In the present study, the potential toxic, cytotoxic and genotoxic effects of three composite nanomaterials were evaluated. The first two were two hybrid layered materials derived from the intercalation of a 'molecular diamond' (adamantylamine, ADMA) in the interlamellar space of graphene oxide and aluminosilicate smectite nanoclay (samples denoted as GO/ADMA and Clay/ADMA, respectively). The third material was a hybrid complex superstructure consisting of 2D nanoclay and 1D carbon nanotubes (CNTs) where CNTs were grown on phyllosilicate nanoclay sheets by the Catalytic Chemical Vapor Deposition method. The latter was functionalized by acid oxidation in order to create carboxyl groups at the surface of CNTs and thus a highly dispersible in polar solvents hybrid derivative (sample denoted as ox(Clay/CNTs). These nanomaterials have a variety of potential applications in the fields of environmental remediation and biomedical applications. The *in vitro* Cytokinesis Block Micronucleus (CMBN) assay was applied in order to assess the genotoxic and cytotoxic potential of the nanocomposites. A treatment with 10, 25 and 50 μg/ml of all composite nanomaterials did not induce micronuclei at significant level as compared to control, showing lack of genotoxicity. However, all nanocomposites showed cytotoxic potential. The Somatic Mutation And Recombination Test (SMART), an *in vivo* assay able to detect the mutagenic and recombinogenic activity of the compounds under investigation, was subsequently employed. *Drosophila melanogaster*, a model-organism for *in vivo* toxicology research, is used in this assay. In order to explore the toxic effects of the three nanomaterials, *Drosophila* third instar larvae were treated with concentrations ranging from 100 to 4000 μg/ml. Data showed that none of the three nanomaterials exhibit toxic activity. To explore their genotoxic potential the higher concentrations of the tested nanomaterials (1000, 2000 and 4000 μg/ml) were used. None of the three nanomaterials induced statistically significant mutagenic events, which indicates absence of genotoxic activity in the specific concentrations and experimental conditions.

TU076
Evaluation of the toxicity of graphene-related nanomaterials on fish cell lines
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The potential benefits of nanotechnology have already been recognized, however, like all new technologies, it raises concerns about the implications for the human health and the environment. While taking advantages of the use of nanomaterials (NMs), their possible adverse effects on biological systems need to be identified. Graphene-related NMs are one of the most attractive NMs from an application perspective, consequently their release into aquatic environments is highly likely. Despite this, only a few toxicological studies have focused on these materials and derivatives. The objectives of this study were to investigate the underlying mechanism of toxicity of carbon nanofibers and graphene oxide on two different cell models including fish hepatocytes (topminnow fish hepatoma cell line, PLHC-1) and macrophages (carp leukocyte cell line, CLC). The particle size distribution of the NMs (and its evolution) in cell culture media was characterized by dynamic light scattering and results showed stable suspensions for all NMs under experimental conditions. Cells were exposed to a concentration range of 0-200 $\mu g\ ml^{-1}$ of NMs for 24 and 72 h and cell viability was assessed by applying on the same set of cells three different cytotoxicity assays (alamarblue, 5-carboxyfluorescein diacetate-acetoxymethyl ester, and neutral red uptake that provide information on the mitochondrial activity, plasma membrane integrity and lysosomal function, respectively). Moreover, interference of NMs with the assay reagents has been tested as well. Some differences in cellular responses to the different types of NMs were observed, however, in most cases toxicity was pronounced only at the highest exposure concentration. In general, both cell lines exhibited time-dependent toxic responses. This is the first study to examine the cytotoxic effects of these graphene-related NMs that are used in a variety of intermediate industrial products. To further examine the ability of these NMs to enter the cell, ongoing research will investigate the possible internalization and intracellular fate of NMs by electron microscopy techniques. This research is supported by the EU's Horizon 2020 research and innovation programme (NanoReg2, Grant Agreement nº 646221).

TU077
Assessment of few layer graphene and graphene oxide effects at the skin level: the role of mitochondria
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The extraordinary physicochemical properties of graphene-based nanomaterials (GBNs) make them promising tools in nanotechnology and biomedicine. However, despite the huge GBNs technologies progress, little is known about their impact on human health. In particular, GBNs cutaneous toxicity remains largely unexplored, despite the skin is one of the feasible human exposure routes to GBNs. Hence, in the effort to evaluate the putative mechanism of toxicity of two GBNs (a few-layer graphene, FLG, and a graphene oxide, GO) towards human skin keratinocytes, their effects at mitochondrial level were evaluated using HaCaT cells. FLG induced a significant reduction of mitochondrial activity after 48 h exposure, whereas GO was effective already after 24 h. FLG was also significantly less potent than GO after 72 h exposure (EC_{50} = 62.8 and 5.4 µg/ml, respectively). Mitochondrial dysfunction seems to be dependent on GBNs-mediated mitochondrial membrane depolarization (MMD): 72 h exposure to the highest FLG or GO concentration (100 µg/ml) increased MMD by 44% and 56%, respectively, an effect comparable to that of the positive control valinomycin (46% at 0.1 µg/ml). However, GBNs-induced MMD increase was not prevented by cyclosporine A (a mitochondrial permeability transition pore inhibitor), suggesting that the effect is due to a mechanical damage induced by GBNs interaction rather than to a biochemically controlled response. To further characterize the mitochondrial damage, reactive oxygen species (ROS) production was evaluated in cells exposed to GBNs for 24 h using different methods (NBT, DCF-DA and luminol assays). A significant concentration-dependent ROS production was induced by both FLG and GO. The kinetic of ROS production was deeper investigated using a time-dependent DCF-DA assay. FLG and GO induced a time- and concentration-dependent ROS production in HaCaT cells, GO being the most active at each exposure time (3-72 h). At 72 h, the highest concentration (100 µg/ml) increased ROS production by 85% (FLG) and 124% (GO), suggesting a role of ROS in mediating mitochondrial dysfunction induced by the two GBNs. Further studies are in progress to characterize the molecular mechanisms of ROS production induced by these nanomaterials. This study was supported by the European Union H2020 Program under grant agreement no. 696656-Graphene Flagship Core1.

TU078
Tissue specific differences of copper oxide nanoparticle toxicity in the marine mussel Mytilus edulis
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The current and foreseen applications of engineered nanomaterials (ENMs) embrace a wide range of technological domains and represent a significant economical fact. One of the most extensively use nanoparticle is the copper oxide nanoparticle (CuO NP) that is present in many commercial products and that will inevitably end up in aquatic environments. In that context, the objectives of the present study is to investigate the signaling pathways implicated in CuONP toxicity in the marine mussel *Mytilus edulis* so as to define signature profile of this NPs in this marine bivalve. Mussels were exposed in laboratory to CuO NP (10 μg/L) or soluble Cu during 24h and changes in mRNA levels of a battery of genes involved in defense, cell metabolism, transport, cytoskeleton were investigated by quantitative RT-PCR analysis. Results showed tissue-specific differences in gene expression between hemocytes and gills due to their difference of role in the marine bivalve. However, some marked effects were observed for mussels exposed to CuO NPs compared to Cu^{2+} on GST, SOD, MT and Actin mRNA gene expressions indicating that CuO NP effect was not solely due to the release of Cu2+ from the nanoparticule form. The overall results allowed to potentially novel molecular targets of CuONP and Cu^{2+} exposures in the mussel. This « omic » template tool will be use in future studies to screen of battery of NPs in the mussel that will allow to create databases for future regulations of the use of nanoparticles.

TU079
Reproductive toxicity via JAK/STAT and TGF-ß pathways crosstalk as potential adverse pathway of UV activated TiO2NPs in the nematode C. elegans: Integrated transcriptomics and metabolomics analyses
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Titanium dioxide nanoparticles (TiO_2NPs) are one of the most widely used NPs, of which catalytic activity is mainly due to photoactivation. Through investigation of toxicity mechanism of nanomaterials, reflecting the real exposure scenario is important for their safe application and use. In this context, phototoxicity of TiO_2NPs was investigated on the nematode *Caenorhabditis elegans* with/without UV activation (i.e. Control, TiO_2NPs, UV, UV+TiO_2NPs). Toxic Mechanism study was conducted using integrated transcriptomics and metabolomics under the exposure condition that caused reproductive toxicity to *C. elegans*. Network analysis was then followed to select involved pathways and selected pathway was experimentally validated with *C. elegans* functional genetic analysis. UV activated TiO_2NPs led significant reproductive and lethal toxicity through oxidative stress to *C. elegans*. DEG analysis from microarray revealed only 4 genes differentially expressed by TiO_2NPs alone, whereas, 3,625 genes by UV alone and 3,286 by UV

activated TiO_2NPs exposure. Pathway analysis suggested possible involvement of JAK/STAT and TGF-ß pathway in phototoxicity of TiO_2NPs, which was further validated by observation of increased gene expression of those pathways. Comparative analysis on *C. elegans* response across UV activation and TiO_2NPs exposure using loss-of-function mutants of genes in these pathway revealed that JAK/STAT pathway as TiO_2NPs specific, whereas, TGF-ß pathway as UV specific. The outcome of our study will contribute significantly to better understand the mechanism of phototoxicity of TiO_2NPs, and in particular, would present a paradigm for elucidation of regulatory signaling cascades with the power of integrated 'OMICS' (transcriptomics and metabolomics) and pathway analysis induced by nanoparticles. Keyword: *Caenorhabditis elegans*, TiO_2NPs, Photoactivation, Multi-OMICS, JAK/STAT and TGF-ß pathways

TU080

Establishing an optimal culture medium and the most responsive viability assay to accurately assess Ag NPs toxicity in primary cultures of E. fetida coelomocytes

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Nanotoxicity studies focused on the effects of Ag NPs on soils are becoming more frequent, dealing most of them with survival, growth and reproduction measurements in invertebrates. Recently, apart from traditional endpoints *in vitro* approaches with earthworm immune cells (coelomocytes) have been developed as cost-effective tools for toxicity assessment of chemicals. These *in vitro* models require an accurate composition of the culture medium to achieve good experimental reproducibility and to ensure that the responsiveness of cells against toxicants is not compromised by culture conditions. The aims of the present work are to select an optimal medium for the maintenance of primary cultures of coelomocytes, and to validate the most accurate viability assay for Ag NPs toxicity assessment. Coelomocytes of *Eisenia fetida* were seeded in 96-well microplates in different media (Leibovitz´s L-15, Basal Medium Eagle-BME- and RPMI-1640) and were exposed to PVP-PEI coated Ag NPs (5 nm), to the coating agent PVP-PEI and to $AgNO_3$ (0, 0.01, 1, 10 and 100 mg /l medium). After 24 h viability was assessed through Neutral Red Uptake (NRU), Cell Proliferation WST-1 and Calcein-AM viability assays. NP size (DLS) and absorbance spectrum were characterised in the three media. NRU and WST-1 assays showed interferences with the exposure media dismissing these tests for an optimal assessment of Ag NPs toxicity. Through Calcein AM viability assay, coelomocytes maintained in L-15 showed higher LC_{50} values than the exposure doses used for both Ag forms (>100 mg/l). In BME and RPMI-1640, the lowest calcein retention was found after Ag NPs exposure (LC_{50} 36.68 and 30.48 mg/l, respectively), followed by $AgNO_3$ exposure (LC_{50} values were around 45 mg/l in both media). Coelomocytes exposed to PVP-PEI in BME and RPMI-1640 media showed a higher calcein retention capacity in comparison to both Ag exposures. Thus, Calcein-AM viability assay occurred to be the most responsive test to assess Ag NPs toxicity and allowed to establish RPMI-1640 as the optimal medium. Moreover, Ag NPs size was stable through time and more sensitive responses were obtained with this medium. Ag NPs were more toxic than $AgNO_3$ and PVP-PEI was discarded as a toxicity enhancer. Culture medium composition is a critical factor for Ag speciation and subsequently for the assessment of Ag NPs toxicity in coelomocytes.
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TU081

The role of reactive oxygen species in the mediation of the toxicity induced by panel of nanomaterials on the freshwater microalgae Raphidocelis subcapitata

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The increased use of nanomaterials in a wide range of products is likely to lead to larger releases of nanomaterials (NMs) into the environment. In this study, the effects of different dispersion methods on the generation of reactive oxygen species (ROS) in the mediation of the toxicity induced by panel of nanomaterials on the freshwater microalga *Raphidocelis subcapitata* were evaluated. *R. subcapitata* was exposed to widely used NMs, silver nanomaterials (Ag NMs) and multi wall carbon nanotubes (MWCNTs) at different sublethal concentrations based on the results obtained from the standard acute growth inhibition tests (10, 20, 45, 90μg/L, for Ag NMs, and 0.85, 1.7, 3.42 and 6.83 mg/L, for MWCNTs). The highest concentration correspond to the obtained EC_{50} value in the standard tests. NMs were dispersed using bath or probe sonication. The ROS production was measured by using the cell permeable indicator 2',7'dichlorodihydrofluorescein diacetate (H_2DCFDA) at different time points (2, 6, 24, 48, and 72h). Cellular esterases hydrolyze the probe to the nonfluorescent 2',7'dichlorodihydrofluorescein (H_2DCF). In the presence of ROS and cellular peroxidases, H_2DCF is transformed to the highly fluorescent 2',7'dichlorofluorescein (DCF). Fluorescence of DCF was measured every 5 min up to 60 min with a microplate reader set at a photomultiplier, with excitation and emission filters of 488 and 525 nm, respectively. After 2h of exposure to the tested Ag NMs, across all concentrations and dispersion method, no significant difference between the treatments was found. Interestingly after 6h of exposure to Ag NMs dispersed using probe sonication, a significant increase in ROS production across all concentrations was noticed. After 24, 48 and 72h of exposure across all tested NMs, concentrations and dispersion method significant increase in ROS production was observed. Based on the above results it is apparent that different approaches used to suspend NMs influence the ROS production pattern. Ag NMs dispersed using probe sonication caused a faster increase in ROS production compared to the Ag NMs dispersed using bath sonication. Different types of sonication affect the physio-chemical properties of NMs such as solubility, size and surface charge, which is likely to lead to different exposure conditions and thus, effects. In further work, the relationship between different physicochemical properties of NMs and ROS production will be investigated.

TU082

Addressing the challenges of emerging contaminants in aquatic systems

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The interest in the environmental effects of emerging contaminants continues to increase due to lack of knowledge on their use, fate and effects, but also the lack of techniques necessary for monitoring their occurrence in the environment. Once such group of emerging environmental contaminants are engineered nanomaterials (ENMs). The interest in these materials is continuing to grow along with the increasing number of applications of nanotechnology. Although availability of data continues to increase, the focus is still on a narrow range of species with little attempts to compare effects across species and how exposures in realistic environmental conditions may affect bioavailability and hazard. In this presentation, the results of exposures of a range of nanomaterials, with different modifications and functionalisations, will be compared across different environmental conditions and species. This presentation will provide a summary of the state of the art on the occurrence of specific ENM effects in biological systems; differentiate cells/tissue injuries due to nano and non-nano materials and between ENMs with similar chemistry but different physical properties, comparing studies of pristine ENMs in static conditions with more realistic scenarios. Results from this work contribute to current developments in the field including the derivation of read-across and cross taxa and endpoints comparisons.

TU083

The effects of biochar on the toxicity of Silver Nanoparticles (AgNPs) to the Annelid species Enchytraeus albidus (E. albidus)

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Silver nanoparticles (AgNPs) are among some of the most widely used nanoparticles. Their anti-microbial and anti-inflammatory properties make them ideal for applications in various consumer products but insufficient information exists on their effects, particularly within the soil environment. The present study investigated the effects of biochar on the toxicity of AgNPs to the species *Enchytraeus albidus* (Annelida). Artificial OECD soil and biochar-amended OECD soil were used to expose *E. albidus* to 5 treatments of AgNPs (0, 5, 25, 125, 625 mg Ag/kg) for 21 days. A suspension of silver nanoparticles known as NM-300K was used for this study. In all AgNPs treatments for both non-biochar and biochar amended OECD soil, reproduction was statistically lower than in the respective controls ($P < 0.05$). However, mortality was only statistically significant at the highest concentration (625 mg Ag/kg; $P < 0.05$) in both soil substrates. When the results were compared, it was found that biochar amendment significantly reduced the toxicity of AgNPs between the highest treatments (625 mg Ag/kg, $P < 0.05$) for both reproduction and survival. A previously unknown 21 day LC_{50} of 833.43 mg Ag/kg for the (NM-300K) used in the study was also generated. The presence of heavy metals in the environment has been a long standing problem, further research could see biochar being used to mitigate the effects of these toxicants during soil remediation processes.

TU084

Sub-lethal effects of nanofullerene C60 on Cladocera quantified by liquid chromatography coupled in a hybrid quadrupole-Orbitrap

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Fullerenes are carbon-based nanomaterials with a particular interest in biomedical research, consumer products and industrial application. The main anthropogenic incidental source of fullerenes is hydrocarbon-combustion, present on industry and combustion process. In this study we observed sub-lethal effects of fullerene C_{60} on daphnids and compared the exposure concentrations with the real concentrations quantified by analytical techniques because the unique physico-chemistry of fullerenes in aqueous matrices requires more than conventional analytical approaches. These combined techniques were important to regard correctly the relation between dose and effect on aquatic organisms. We exposed one organism per replica to two different suspended solutions of fullerene C_{60}, 6.25 and 25% concentrations (102.5 and 179.1 μg/L) and to a control treatment. We monitored the development of *Daphnia similis* neonates until become females and their posterity until the 12th day after birthday. Endpoints as female's fecundity, hopping frequency and survival were studied. First, to define the exposure sub-lethal concentrations, lethal effects was observed and calculated in 20% of the exposed population. Consequently was possible to choose two representative concentrations to study the sub-lethal effects. To the analytical study a specific column with pyrenyl-propyl stationary phase was employed for the separation of the analytes, showing an improved retention in comparison with conventional C18 columns and a satisfactory separation from the potential matrix interferences. The ionization was carried out with a hybrid atmospheric pressure chemical ionization/atmospheric pressure photoionization source (APCI/APPI) working in negative mode, and the detection was carried out with a Q-Exactive mass spectrometer with a hybrid quadrupole-Orbitrap analyser. The LC_{20} were 27,7% (179.1 μg/L) of the standard solution (2793,2 μg/L). Therefore we chose the nominals concentrations 6,25%(low effect) and 25% (high effect) to expose the organisms on the chronic toxicity bioassays. The bioassays concentrations range between 6,25% and 100%. The fecundity of 12,5; 14,5 and 5,5 neonates/female was observed on the control, low and high concentrations, respectively. In both toxicity exposures, 33% of the females died and the number of hopping of each female was 4,9; 4,5, and 3,5 hopping in 10 seconds, respectively. The exposure concentrations were confirmed by the analytical technique proposed.

TU085

The use of proteome profiling to detect potential biomarkers for monitoring the immunotoxicity of engineered nanoparticle

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Introduction Engineered nanoparticles are a group of chemicals with unique characteristics. Due to their superior chemical and physical properties, nanoparticles are used in energy trapping and storage devices, pharmaceuticals, in clothes as anti-microbial agents, in electrical components, in ultra-lightweight super-strength materials for vehicles and space exploration, and many others. Due to the rapid increase in the production and application of nanoparticles, concerns are being raised regarding risks that these particles may pose to human and environmental health. Conflicting results have been published for toxicity of specific nanomaterials, especially for silver and carbon nanoparticles, This may be due to the fact that species vary in their sensitivity for specific nanoparticles, or could also be due to the use of inappropriate biomarkers to evaluate events. Proteome profiling can be used to monitor a range of biomarkers in a single experiment. **Aim of study** The aim of this study was to investigate the effects of carbon nanodots, amine capped carbon nanodots, silver nanoparticles and graphene oxide nanoparticles on the cytokine and chemokine proteome profile of cultured RAW264.7 macrophage cells. **Methods** Raw264.7 cells were cultured in 24 well culture trays. Upon reaching approximately 60 % confluence, cells were treated overnight with various concentrations of the nanoparticles. At the end of the treatment the culture supernatants were removed and screened for 40 different cytokines and chemokines using a mouse cytokine antibody array (R&D Systems). An aliquot of the supernatant was also screened for nitric oxide and interleukin 6 as biomarkers for inflammation. The cells were evaluated for cytotoxicity using the WST cytotoxicity assay. **Results** Results obtained showed that the nanoparticles used were not cytotoxic. Initial data also indicated that the freshly prepared nanoparticle suspensions have no effect on IL-6 and nitric oxide synthesis. However, cytokine proteome profiles show that the nanoparticles upregulated some of the inflammatory cytokines such as MIP-1β and TNF-1α. The data also shows that storage of particles modulated the effects of nanoparticles on the immune system. **Conclusions** Investigations using traditional *in vitro* toxicity and inflammatory activity tests together with proteome profiling will give a more holistic picture of potential adverse effects posed by nanoparticles.

TU086

Impact of acute and chronic inhalation exposure to PbO nanoparticles on mice

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Although widely used, nanoparticles can still represent a risk since there is little knowledge especially when concerning an impact resulting from the differences between short and long term exposures. This study is therefore focused on both acute and chronic exposures to lead oxide nanoparticles (PbO NPs) that are important materials for industry. Mice were exposed to PbO NPs in whole body inhalation chambers for up to 72 hours in acute experiment (4.05×10^6 nanoparticles/cm^3) and for up to 11 weeks in chronic experiment (3.83×10^5 nanoparticles/cm^3 in lower and 1.93×10^6 nanoparticles/cm^3 in higher exposure group). Transmission electron microscopy documented distribution of nanoparticles into all studied organs including lung, liver, kidney and brain. Analysis of Pb content in tissues by electrothermal atomic absorption spectrometry showed gradual accumulation of lead in all studied organs with increasing exposure duration. Histological analysis documented numerous alterations in morphology and tissue damage. Modulations of oxidative stress parameters including oxidized and reduced glutathione, glutathione-S-transferase activity and lipid peroxidation were detected especially after extended exposure. This study was supported by the Czech Science Foundation grant No. P503/11/2315 and P503/12/G147 and by project LO1214 funded by the National Sustainability Programme of the Czech Republic.

Aquatic and Terrestrial Plant State-of-the-art Research linking ecotoxicology and exposure of chemicals (P)

TU087

Ecosystem services approach to pesticide risk assessment and management of non-target terrestrial plants: updated summary and recommendations from SETAC Europe workshops

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Two SETAC Europe workshops (April 2014, Sept 2015) were organised to discuss how to perform and implement higher tier studies or how to use them to refine the risk assessments for Non-Target Terrestrial Plants (NTTPs). The recommendations from the first workshop related to specific protection goals, risk assessment scheme and mitigation. The workshop participants agreed that the type and relative importance of ecosystem services provided by NTTPs differ between in-field and off-field areas. They also identified some areas for further evaluation and analysis that would reduce uncertainty in the risk assessment. In particular, the protectiveness of standard test species for wild species and the protectiveness of regulatory endpoints for reproductive endpoints were evaluated. At the second workshop, besides the outputs from the first workshop, EFSA's Scientific Opinion on the risk assessment for NTTPs was considered. Comparative analysis of wild species versus crop species revealed that testing crop species is protective of wild species. In addition comparative analysis of reproductive and vegetative endpoints indicates that the former are on average less than a factor of 2 more sensitive than the vegetative endpoints when comparing the same point estimate (i.e. ER_{10}, ER_{25} or ER_{50}). It should however be noted that such a factor could be within variability of species sensitivity. In the comparison of the endpoints currently used in the risk assessment of NTTPs (ER_{50} for juvenile plants) to ER_{10} for reproductive effects on mature plants, the difference in endpoints ranged between 6.25× and 8.68×. At the second workshop a framework for higher tier risk assessment for NTTPs was also proposed. Furthermore it was considered that mitigation measures described in MAgPIE are appropriate for NTTPs and that compensation for possible in-crop effects on ecosystem services should be defined by risk managers in light of the specific protection goals. For this, several pieces of legislation may be relevant in concert with the Pesticide Regulation 1107/2009.

TU088

Proceedings in the establishment of field studies as a potential higher tier option to refine risk assessment for non-target terrestrial plants

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During the regulatory approval process for pesticides, potential side-effects to non-target terrestrial plants (NTTP) need to be assessed. Routine testing with NTTPs is done in highly standardized greenhouse studies under somewhat conservative assumptions. If the obtained results are not sufficient to demonstrate acceptable risk, higher tier testing may be needed. Field studies are frequently discussed as higher tier options but so far no agreed method has been established. Here we present results of pilot field studies done in order to establish a higher tier method for refining risk assessment for NTTPs. A homogeneous distribution of plant species within and across replicates in a study field is an important prerequisite for data evaluation in the risk assessment paradigm. Hence, commercially available seed mixtures were evaluated as a potential source for establishing higher tier study sites. In preceding field studies (2014 & 2015), it was found that both seed mixture and growth area greatly influence the distribution of species and homogeneity of replicates. Two seed mixtures that showed potential in previous years were further tested in 2016 by comparing their growth in three different locations. To overcome the severe problem of emergence of species from the soil seed bank, specific farming measures were taken at all three sites. Vegetation cover was assessed according to the Londo scale in five replicates per seed mixture during the growth season (June to September). Thoughtful choice of seed mixture and appropriate preparation of the sites improved homogeneity of plant distribution. The Londo scale proved to be more suitable to reflect changes in vegetation cover more accurately than the previously used Braun-Blanquet scale. Nevertheless, small differences in vegetation coverage in between replicates seem to be unavoidable. In addition, the utilized measures reduced emergence of plant species from the soil seed bank in comparison to 2014/15. Even though more than 20 species that were not sown were detected in the trial sites, the vast majority of these species were only present in low or negligible abundance while all species from the seed mixtures were able to germinate in all locations. Nevertheless, abundance of the sown species differed greatly in between the three study locations, emphasizing the importance of weather and soil conditions. Overall, this study can be seen as an important first step in establishing field trials for NTTPs.

TU089

Phytotoxicity Assessment of Methanol and Toluene Introduced into Paddy Field by Chemical Spill on Rice (Oryza sativa)

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In Korea, there are many soil contamination cases by methanol (CH_3OH) and/or toluene ($C_6H_5CH_3$) by chemical incidents. Methanol and toluene can be introduced into the agricultural field by groundwater flow or spill. Rice (*Oryza sativa*) is the most consumed crop in Korea, and rice paddy fields are distributed throughout Korea. Due to the shortage of methanol and toluene toxicity data on rice, it is not easy to assess their accurate impact on rice when chemical incidents occur. In this study, phytotoxicities of methanol and toluene on rice were assessed in laboratory scale. The phytoxicity tests were performed based on OECD guideline 227. Rice seeds were germinated in the non-contaminated bed soil and incubated for 4 weeks. After incubation, the rice seedlings were transplanted in the test soil homogeneously mixed with the methanol or toluene. Concentrations of methanol or toluene in test soil were designated 7 levels including control. The seedlings were incubated in the contaminated and control soil for 4 weeks. The biomass, elongation, and visible detrimental effects of rice were observed during incubation period. The toxicity values (LOEC and EC_{50}) on the basis of the measured endpoint were calculated using SAS 9.3 software. The results of this study will provide the preliminary information on the toxicological risks of rice by methanol and/or toluene introduced by chemical spill. ***Acknowledgement***: This subject is supported by Korea Ministry of Environment(MOE) as "The Chemical Accident Prevention Technology Development Project."

TU090

Phytotoxicity of endocrine disrupting chemical (Bisphenol A) on mungbean and rice

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Bisphenol A is used for raw materials of synthetic resins, coating agents of aluminum can and receipt paper. Bisphenol A could be released into terrestrial ecosystem via several routes; however, very limited data for toxicity of bisphenol A on plants were reported. This sutdy evaluated acute and chronic phytotoxicity of Bisphenol A using crop plants rice (*Oryza sativa*) and mungbean (*Phaseolus radiates*). We measured shoot growth and photosynthesis factors (Area of leaf) in acute toxicity test. The shoot growth, root development, photosynthesis factors, surface area of leaves, length of stoma and chlorophyll contents were measured in the chronic toxicity test. The 14d-EC_{50} and NOEC were estimated and the 21d-EC_{10} was also estimated. We observed that Bisphenol A inhibited growth and photosynthetic performance of plants and damaged stoma. In addition, chlorophyll contents were effected by bisphenol A. This study demonstrated that phyto-toxicity of bisphenol A on dicotyledoneae and monocotyledoneae, and suggested toxicity values of bisphenol A. *This study was funded by the Korea Ministry of Environment (MOE) as the Environmental Health Action Program (RE201603033), and Graduate School of Specialization for managing information of chemical risk.*

TU091

Influence of nitrogen source on wheat plant rhizosphere pH and uptake of lamotrigine

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Irrigation with treated wastewater and soil amendment with biosolids are increasingly common practices in agricultural systems, and result in exposure of crop plants to a variety of ionizable organic contaminants (IOCs). Crop plants may take up and bioaccumulate IOCs, but there are many gaps in our understanding of the variables that control their phytoavailability. Soil pore water pH determines how much of an IOC is present in its neutral form. However, plants can change the pH of the soil immediately surrounding their roots up to 2 units in either direction by secreting H^+, OH^-, HCO_3^-, and organic acids in response to nutrient availability. To our knowledge, the influence of rhizosphere conditions on the availability of IOCs to plants has not previously been studied, though root exudates can affect the partitioning and bioavailability of other contaminants. We investigated the effects of different nutrient solutions on wheat plant rhizosphere conditions and uptake of lamotrigine, an anti-epileptic drug (pK_a = 5.7). We grew wheat plants in a system designed to be able to measure conditions in the growth media directly surrounding the roots. Plants were grown on nutrient solution that contained either only nitrate or ammonium and nitrate as the nitrogen source. Though the initial pH was the same for both treatments, by the end of the exposure period, the plants grown on only nitrate had a significantly higher rhizosphere pH and significantly more lamotrigine in their aerial tissues than the plants that were also grown on ammonium. The lamotrigine concentration in the aerial tissues correlated with the neutral fraction of lamotrigine at the rhizosphere pH. The implication of these results on the bioavailability and accumulation of other IOCs will be discussed.

TU092

De novo transcriptome sequencing and gene expression profiling of Phoenix dactylifera L. under cadmium stress

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The development of transcriptomic responses helps plants survive and resist to abiotic stresses. However, little is known about stress responses to abiotic stress of non-model plants especially for *Phoenix dactylifera* species. To get a deeper insight into its molecular mechanisms of Cd tolerance, we report an annotated transcriptome assembly for *P. dactylifera* cv Deglet Nour. From total RNA extract, 37,049 unique Illumina RNA-seq reads were produced and used in the transcriptome assembly. The draft transcriptome assembly consists of 6789 contigs and 17.285 singletons with a means length of 858 and 1.042 bp, respectively. The final assembly was functionally annotated using Blast2GO software, allowing the identification of putative genes controlling important agronomic traits such as Cd transporter coding genes and gene involved in glutathione metabolism. There are several metal transporters families that can contribute to the resistance of plants to heavy metals including PdATP binding cassette (*PdABC*), Natural resistance-associated macrophage proteins (*PdNramps*), *ATPase* family and *PdMATE* efflux family in addition to Cd chelator genes like phytochelatin synthase (*PdPCS*) and metallthionein (*PdMT*). The expression of in silico founded orthologous genes, was monitored by qPCR expression analysis. Expression levels were measured in isolated explants of date palm cv Deglet Nour exposed to Cd during various time periods (up to 2 months). Each Cd-responsive gene was expressed in a specific manner along metal concentration variations during two months of treatment. The Cd treatment induced the expression of target genes in the first 20 days of exposure. Marked transcript accumulation of *PdPCS*, *PdATPase*, *PdMATE* and *PdPDR* has been shown as well as up-regulated *PdMT* and *PdABC* expression levels. Increasing metal time exposure further induced genes expression under 0.02 and 0.2 mM Cd. However, a significantly important decrease of the level of expression was observed at the end of the treatment for *PdABC*, *PdMT* and *PdATPase*.The *P. dactylifera*transcriptome highlights the activation of a large set genes potentially involved in metal stress response and/or Cd detoxification.

TU093

Verifying the protection level for aquatic primary producers in the first tier of the risk assessment scheme for plant protection products

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The environmental risk assessment (ERA) for plant protection products (PPP) in the EU follows a tiered approach. For compounds with an herbicidal Mode of Action and plant growth regulators, tests with a green alga, a non-green alga and *Lemna* sp. are required in the aquatic first tier of the risk assessment scheme. If *Lemna* sp. is not sensitive, additional tests for other aquatic macrophytes should be performed. Before 2015, the lowest endpoint among ErC50, EbC50 or EyC50 (growth rate, area under the curve, yield) could be used. Since 2015, the aquatic Guidance Document (AGD) (EFSA Journal 2013; 11(7) 3290) recommends to use preferably the ErC50. Since EyC50 and EbC50 values are often lower than ErC50, there is a shift towards a reduction in the margin of safety achieved as shown by Swarowski et al, 2015 (SETAC Europe 2015) and Arts and van Wijngaarden (poster SETAC Europe 2016, Nantes). The overall question to clarify is whether the level of protection reached when using ErC50 (in combination with the standard assessment factor of 10) remains sufficient. To do so, the AGD *reports that "... a proper calibration between different tiers (higher and lower tier data) ... should be performed in the future".* The work presented here contributes to this calibration/ validation of the tiered risk assessment approach by comparing the standard first-tier effect assessment for herbicides and fungicides with an herbicidal mode of action, with results of micro-/ mesocosm studies. We thus evaluate the protective value of the Tier-1 effect assessment using EC50 and if possible EC10 values of standard test algae and macrophytes based on either the growth rate endpoint or the lowest available endpoint for growth rate or AUC/yield. To do so, these Tier 1-Regulatory Acceptable Concentrations (RACs) are compared to the higher Tier-RACs for the threshold option as derived from micro-/ mesocosm studies. Similar work of Arts and van Wijngaarden (poster SETAC Europe 2016, Nantes) indicated that when using ErC50 the level of protection remains sufficient. The results of the current study will enable to investigate if these preliminary conclusions are confirmed based on a more extended data set.

TU094

Recovery of Lemna minor following an exposure to the sulfonylureas

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Pesticides release to surface waters usually takes place in pulses between which a recovery can occur. This study investigates the potential of common duckweed (*Lemna minor* L.) to recover following a 7 day exposure to sulfonylurea herbicide amidosulfuron. The exposure to amidosulfuron significantly reduced the growth of *L. minor*, altered the content of photosynthetic pigments and induced membrane lipid peroxidation. Following transfer to a clean growth media, the plants exposed to high concentrations of amidosulfuron did not manage to recover. *L. minor* exposed to relatively low levels of amidosulfuron showed a potential to recover their new fronds production, whereas biomass recovery was less efficient and after recover phase the final biomass of the exposed plants was below their initial values.

TU095

A proposed ring-test protocol for the emergent macrophyte, Glyceria maxima, in a water-sediment system

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Under EU pesticide regulation, regulatory tests are required for the aquatic macrophyte, *Lemna*, and two algal species for herbicides and plant growth regulators. Data requirements introduced under EU Directive 1107/2009 stipulate that further tests may be required for compounds which show selectively higher toxicity to either dicotyledonous or monocotyledonous plant species in terrestrial plant tests. In these cases, the recommended dicot and monocot species are *Myriophyllum* and *Glyceria*, respectively. OECD Test Guideline 239 for testing *Myriophyllum spicatum* in a water-sediment system was adopted in September 2014. The general principles of this test system are applicable to many aquatic plant species and, in 2014, a workgroup was formed to facilitate adaptation of this protocol for testing the emergent, reed grass, *Glyceria maxima*. Since this time, 15 laboratories have expressed interest in participating in a ring-test of this protocol and completed a survey of intended plant propagation methods. Results of this survey confirmed difficulties with the use of seedlings as test material, such that the protocol now recommends use of rhizome-propagated plants. The workgroup has identified the first test substance and laboratories are beginning the first tests in Autumn 2016. The first series of tests is designed to evaluate whether a test duration of 14 or 21 days is required and to gain information regarding control variability across several assessment parameters, i.e. shoot height, leaf length, fresh weight and dry weight. Available data will be presented to address these issues.

TU096

Suitability of several dicotyledonous macrophytes as additional test species for the risk assessment refinement

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The species sensitivity distribution (SSD) method is an often used approach in the tier 2 risk assessment (RA) of plant protection products (PPP) in which additional toxicity data of 8 potentially sensitive species can be provided by the applicant(EFSA 2013). In general, a group of species for which the PPP is indicated should be chosen. In the case of an herbicide, species of macrophytes and/or algae should be tested for a SDD. However, even within the group of macrophytes the sensitivity to PPPs can be distinct different based on the class of the plant. For example, the substance group of herbicides acting as auxins are only effective on dicotyledonous plant species at low concentrations. Therefor it would be appropriate to test only dicotyledonous macrophytes for a SSD as refinement method in the aquatic RA of auxins. In this study, we tested several dicotyledonous macrophytes in a multispecies test system in order to gain information on their suitability as additional test species in a SSD approach. In total, 15 submerged and riparian aquatic species were tested. For that, one individual of each test species was planted in a glass beaker filled with 3 layers of sand, commercial pond soil (peat basis) and sand. In total, 10 individuals per species were tested by placing the beakers in an artificially illuminated indoor microcosm system filled with water. Growth rates and coefficient of variation were determined on several occasions for several endpoints during the 28 d experimental phase. Suitability of the different dicotyledonous macrophytes for the inclusion in a SSD approach will be presented.

TU097

Intraspecific variation in the sensitivity of two macrophyte species to copper contamination

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Copper is an essential trace element, broadly used in industries and agriculture, and therefore found at high concentrations in some aquatic environments. Macrophytes play key roles for aquatic ecosystems as primary producers: the way they respond to pollution may impact the whole ecosystem dynamics as they are strongly involved in biogeochemical processes and they interact with many organisms. Thus, the importance of the different sources of variation in their sensitivity to contamination needs to be assessed to be able to predict impacts of pollution on aquatic ecosystems and their resilience. The aim of the present work is to improve our understanding of intraspecific variations in the sensitivity to copper exposition of two macrophyte model species, Myriophyllum spicatum and Lemna minor, by using at least three different clonal strains for each species. Four single-species tests were performed, two for each species. Tests were carried out following OECD protocols as close as possible. Copper toxicity was assessed through a range of concentrations from 0 to 1.25 mg/L for each experiment. Three endpoints were measured: maximum quantum yield of PSII (Fv:Fm), fresh weight and shoot length or frond number for M. spicatum and L. minor, respectively. Plants were significantly impacted from 0.25 mg/L of copper for all species and strains considered, as well as for all endpoints measured, except for the length of M. spicatum which was not affected by copper exposition. At 0.25 mg/L of copper, inhibition of growth rate based on fresh weight ranged from 54 to 83% for L. minor and from 47 to 117% for M. spicatum. These differences among strains were significant for M. spicatum but not for L. minor. Myriophyllum spicatum also showed significant differences among strains for maximum quantum yield of PSII (Fv:Fm).\nAll strains of M. spicatum were acclimated the same way (at least 8 weeks prior to the experiments), suggesting that genotypic differences, possibly resulting from local adaptation, drive these phenotypic differences and are involved in intraspecific variations in plant sensitivity to copper. Further investigations are required to determine whether these intraspecific differences in M. spicatum sensitivity to copper, although not very high, could lead to differential fitness among strains in contaminated environments.

TU098

A proposal of novel ecotoxicity test method using automatic analysis of color change in the green alga Ulva pertusa Kjellman(Chlorophyta)

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A bioassay based on visual inspection of reproduction of the aquatic green macroalga, *Ulva pertusa* Kjellman, was developed and used to assess the hazards of different organic and inorganic chemicals and elutriates of sewage or waste sludge. However, this method employs a technique to quantify percentage reproduction based on thallus color changes for the progression of reproduction, which is liable to subjectivity relating to skill of personnel and variability of instruments etc. This study was initiated to develop a more objective way to measure reproductive state of the seaweed. The newly used method was to stain the *Ulva* thallus followed by image analysis of the stained (sporulated) area. After 96 h exposure to toxicants, *Ulva pertusa* Kjellman disks were stained with 0.5% Evans blue for 15 min before taking measurements of stained areas. In addition, coverslips were placed on the bottom of the petri dishes to ascertain whether spores were released since ambiguity exists in the color between fully emptied thalli after spore release and dead thalli. The sporulation percentage was then calculated by the stained area over the whole thallus disk area. EC_{50} values obtained from the original and newly developed method were similar: 0.1166 vs 0.1437 $mg \cdot L^{-1}$ for Cu, 0.0368 and 0.0629 $mg \cdot L^{-1}$ for diuron, 0.1955 and 0.2047 $mg \cdot L^{-1}$ for atrazine, respectively. The *Ulva* reproduction inhibition test method with heightened objectivity has world application since *Ulva pertusa* Kjellman has a wide geographical distribution and species have similar reproductive processes.

TU099

SETAC Plants Interest Group

S. Loutseti, DuPont De Nemour Hellas S.A.

Effects and ecological consequences of aquatic exposures to particulate materials from the nano- to macro- scale (P)

TU100

Aquatic toxicity and bioaccumulation of sparingly soluble manufactured particulate substances

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Aquatic toxicity tests and risk assessment frameworks were designed based on existing infrastructure and engineering controls, which limit aquatic exposures to substances as undissolved particulates. Thus for most conventional substances and associated life cycle stages, the presumed exposure modes involve contact of aquatic organisms with a fully dissolved concentration of the toxicant. The terminology currently used to describe aquatic hazard and risk largely reflects the assumption that as the substance partitions into biota the external dissolved concentration corresponds to an internal dose. However, aquatic exposures to dispersed particulates do not generally involve a state of equilibrium partitioning. Therefore, the TF will explore whether the current terminology and metrics used to describe hazard and risk are adequate to characterise physical effects from undissolved particulate substances. Language and procedures may need to evolve to better reflect the dynamic nature of particulate exposure modes and to adequately describe their associated risks. The purpose was to examine circumstances when studies conducted under existing standardised testing procedures are appropriate for use in risk assessment and clear guidance for when particulate-specific (including nanoparticulate) tests and procedures may be warranted, along with a scientifically sound approach to link measured effects to risk assessment goals. The used methodology was to analyse the literature for case studies (among them silver, gold, Polyethylene or soluble liquids in order to identify possible physical effects based on a reading grid to consistently analyse the retained references. The first results and conclusions of this study will be presented in the poster

TU101

Toxicity of nano metal oxides on two algae, Chlorella vulgaris and Scenedesmus demorphus

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Taking into consideration the increasing application of nanoparticles resulting to their entry into industrial and non-industrial wastes makes necessary to investigate potential effects of these materials on aqueous environments. One of these common compounds is zinc oxide nanoparticle which as a ceramic absorbent material has different applications. In this research, the effect of zinc and tin oxide nanoparticles is investigated on two algae species *Chlorella vulgaris* and *Scenedesmus demorphus*. The appropriate experimental concentrations were determined by performing range-finding tests, and the effect of each concentration of ZnO and SnO_2 nanoparticles was studied during three time periods of 24, 48 and 72 hours against the control in 5 treatments and three replicates for each. The experiment was performed based on OECD method. The number of algae cells was daily counted. Data analysis was performed using Probit analysis and the amounts of EC_{10}, EC_{50}, and EC_{90} were calculated. The sensitivity of *Chlorella* species to ZnO nanoparticle was much more than *Scenedesmus* species: 72h EC_{50} value was 0.01 mg/l for *Chlorella* and 0.09 mg/l for *Scenedesmus*. But, *Scenedesmus* species was more sensitive to SnO_2 nanoparticle than *Chlorella*: 72h EC_{50} is 131.28 and 2.42 mg/l for *Chlorella* and *Scenedesmus*, respectively.

TU102

Effects of metal oxide nanoparticles on the aquatic plant Spirodela polyrhiza

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Nanoparticles, in particular metal oxide nanoparticles, have found extensive usage in a wide range of services and industries. Subsequently, they can be released into environment and finally end up in water bodies[1,2]. That may suppose a potential risk to aquatic environment[3], exerting toxic effects at the level of cells, tissues or the whole organisms. Hydrophytes represent an essential base component in aquatic ecosystems and could play a crucial part in the fate and transport of nanoparticles to higher trophic levels through the food chain. Despite the significant research activities in phytotoxicity area, the aquatic plants response to metal oxide nanoparticles is still poorly known. The present study evaluate the toxicological effects of titanium dioxide (TiO_2), magnetite (Fe_3O_4) and cerium oxide (CeO_2) on *Spirodella polyrhiza* by exploring concentration-dependent effect and changes induced at 24, 48 and 72h of exposure. Seed germination evolution and fronds morphology were used as endpoints to assess the phytotoxicity for NP concentration from 1.25 to 1000 mg L^{-1}. The particle size and the ζ-potential of NPs in the culture media were also measured to analize the relation between agregation profile and the observed toxicity on duckweed. The obtained results reveal toxic effects of TiO_2 and Fe_3O_4 nanoparticles leading to growth reduction and significant changes in the morphology of fronds. Both NPs caused drastic damage in fronds showing at high concentration a clear decoloration. In contrast CeO_2 did not produced significant negative effect. **References** [1]Neale P.A., Jamting A.K., O'Malley E., Herrmann J., Escher B.I. 2015. Behaviour of titanium dioxide and zinc oxide nanoparticles in the presence of wastewater-derived organic matter and implications for algal toxicity. Environ Sci Nano. *2*:86-93. [2]Sahle-Demessie E., Changseok H., Amy Z., Bill H., Heidi G. 2016. Interaction of engineered nanomaterials with hydrophobic organic pollutants. Nanotechnology 27:284003. [3]Oukarroum A., Barhoumi L., Samadani M., Dewez D. 2015. Toxic effects of nickel oxide bulk and nanoparticles on the aquatic plant Lemna gibba L. Biomed Res Int. 501326. Acknowledgement- This work has been financed by the FP7-ERA-Net Susfood, 2014/00153/001 No. 291766, the Spanish Ministry of Economy and Competitiveness, CTM2013-45775-C2-1-R (MINECO/FEDER EU) and the Comunidad de Madrid Network S2013/MAE-2716

TU103

Combined effects of nanomaterials and pollutant mixtures - investigations on zebrafish embryos

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Engineered nanomaterials (ENMs) are released in different environmental compartments due to anthropogenic activity, which may result in co-exposure of organisms to other pollutants e.g. pesticide or pharmaceuticals. Thus, with respect to the assessment of ecotoxicological effects posed by these mixtures, several questions arise: How likely are physico-chemical interactions between ENMs and pollutants in the aquatic environment? Can interaction modify bioaccumulation of either substance? Does co-exposure influence toxicity outcome? Accordingly, the aim of this study was to shed light on potential interactions and their effects in organisms caused by nanomaterials mixed with environmental chemicals. For this purpose, binary mixtures of fullerenes (C_{60}) with different organic compounds with a $\log k_{ow} < 4$ (e.g. 2,4-Dinitrophenol), were prepared. The carbonaceous nanomaterial was chosen because of its low toxicity to zebrafish embryos and the expected high adsorption capacity. Selecting chemicals, we considered: (I) stability in the exposure medium, (II) sufficient solubility avoiding the use of co-solvents, (III) moderate toxicity towards zebrafish embryos. First, the interaction between fullerene and the chemical compound was studied by HPLC analyses of the chemical content of single component solutions as well as mixtures. Results showed no interaction of the selected chemicals with C_{60}. Instead we observed that rapid clustering and surface oxidation of fullerenes dramatically reduced the interaction with the substances. Second, the effect of mixtures of fullerene and organic chemicals on zebrafish embryos was assessed. The simultaneous presence of C_{60} did not change the effect of chemicals on zebrafish embryos, hence no combined effects were observed. Even though the particle clusters were observed to accumulate on the chorion surface, the uptake of chemicals into the embryos seemed unaffected. The aim of future studies will be to confirm that the uptake of chemicals is indeed unaffected by C_{60}. In addition, it remains to be clarified, how other nanomaterials behave in mixtures with chemicals occurring in the aquatic environment.

TU104

Effects of ingestion of antimicrobial nanoparticles on gut health in rainbow trout.

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Incorporation of engineered nanoparticles (NPs) into consumer products is increasing rapidly and the release of these particles into the aquatic environment with exposure of organisms, such as fish, is of rising concern. A relevant exposure route of NPs by organisms is through ingestion. The antimicrobial activity of some of the most frequently used NPs such as Ag- and Cu-NPs can have negative effects on the natural occurring microbiota of beneficial/protective bacteria within the digestive system of organisms. A healthy endogenous gut microbiota is important for overall organism health and has implications on immune system function and pathogen resistance. Our objective was to investigate effects of ingestion of food containing NPs (Ag-NPs, Cu-NPs) or bulk material controls ($AgNO^3$, $CuSO^4$) on gut health in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to 50 mg metal (as NP or bulk material) per kg feed (or control feed). After exposure for 42 days, half the fish were challenged with the bacterial pathogen *Yersinia ruckeri*, the causative agent of enteric redmouth (ERM) disease in rainbow trout. Fish were sampled after 49 and 56 days for assessment of gut microbial community (by Illumina 16s rRNA gene amplicon sequencing and analysis in Mothur) and gut health (gene expression by quantitative reverse transcription PCR and histopathology). We found that dietary exposure to Ag-NP and Cu-NP (or bulk controls) did not affect growth or survival, but initial evaluation of gut microbiota suggest that feed treatments do change the abundance of various bacterial genera. There were no feed-treatment related differences in pathogen infection, appearance of clinical signs of ERM, or fish mortality. The histopathological evaluations, gene expression analysis and a more in depth analysis of the effects on gut microbiota is currently ongoing.

TU105

Environmental behaviour, bioaccumulation and genotoxicity of iron oxide (maghemite) nanoparticle in Poecilia reticulata

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Iron oxide nanoparticles (IONPs) are designed and used to protect the environment through pollution prevention, treatment and clean-up of contaminated sites (nanoremediation) and wastewater treatment. However, their behaviour and fate in the aquatic environment and ecotoxicity on aquatic vertebrates are unclear. So, the aim of this study was to produce IONPs (maghemite) coated with citrate and performer its characterization in terms of individual shape, crystallite size, hydrodynamic diameter, surface charge and aggregation kinetics in freshwater, as well as to investigate the bioaccumulation and genotoxic effects in the guppy *Poecilia reticulata*. IONPs were produced by aqueous precipitation and characterized using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Electrophoretic Light Scattering (ELS). Fishes were exposed to IONPs at 0.3 mg L^{-1} during 21 days using reconstituted water and the animals were collected at the beginning of the experiment and after 3, 7, 14 and 21 days of exposure. The total iron accumulation in the freshwater and fish organs (gills and liver) was determined by atomic absorption spectrophotometry, while the genotoxicity was evaluated in terms of DNA damage (comet assay), micronucleus (MN) and other erythrocyte nuclear abnormalities (ENA) frequency. TEM results demonstrate crystalline and rounded IONP with an average size of 3.97 ± 0.85 nm. The DLS and ELS analysis showed that the IONPs has low hydrodynamic diameter and high surface charge in ultrapure water (14.11 ± 0.2 nm; -51.1 ± 7 mV) compared to reconstituted water (21.4 ± 0.39 nm; -19.5 ± 6.5 mV), indicating that the ionized citrate groups confer negative surface charge of IONPs in both aqueous medium, confirming the tendency of these NPs to aggregate/agglomerate due to low repulsion forces. *P. reticulata* uptake and accumulated the IONPs in an exposure time and organ dependent patterns. Results showed that the IONP accumulation in *P. reticulata* induces genotoxic effects (DNA damage) and changes the MN and ENA frequency compared to unexposed fish according to the exposure time. Overall, this study showed that the behaviour and fate of IONPs may affect their bioavailability and bioaccumulation in the aquatic environment. Results indicated that the *P. reticulata* erythrocytes are a suitable model for assessment the ecogenotoxic effects of engineered nanoparticles used in nanoremediation approaches.

TU106

Impacts of fine particle mine tailings on early life stages of cod

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Mineral industries produce millions of tons of mine tailings waste and in Norway the common practice is to deposit tailings waste by coastal submarine dispersal. Mine tailings slurry include a fraction of fine particles < 100 μm and can have elevated levels of heavy metals from incomplete ore mineral recovery. Prolonged suspension of small particles in the water column may bring them into contact with locally spawned pelagic fish eggs, including commercially valuable Atlantic cod. The objectives were to expose newly-fertilised cod eggs to suspended mine tailings particles from the proposed Nussir ASA copper mine in Finnmark, Norway, and assess their survival, development, hatching, and molecular markers for stress, genotoxicity, and epigenetic changes. Cod eggs were exposed to concentrations within authorised permit limits (1-3 mg/l) of suspended mine tailings particles in flow-through aquaria. Increased concentrations of particles in the water (Beckman Multsizer Analyzer) correlated with an increase in water Cu concentrations, indicating some desorption of bioavailable Cu from particles into the water. Higher concentrations of particles were detected (scanning electron microscopy, SEM) on the chorion surface of the high exposure treatment eggs after 11-d exposure; however, elemental analysis (SEM and additional electron dispersive spectroscopy) of the particles could not diffinitively identify particles of mine tailings origin. There was no adverse effect of mine tailings exposure on embryo mortality, and a slight but significant elevation in larval mortality after hatching, suggesting a a protective effect of the chorion on the embryos and increased stress when in direct contact with particles after hatching. There were no differences with treatment in timing of hatching, cardiac activity, and embryo and larvae morphometrics. There was a treatment-dependent up-regulation of selected stress marker genes (*hsp*, *cyp1c1*) but no indication of activation of metallothioniens (*mt* gene) or markers for genotoxicity (*gadd45* and *rad51*). Gene markers for DNA and histone methyltransferases did show treatment-related up-regulation; however, the long-term implications of changes to epigenetic control of DNA in response to environmental stressors such as mine tailings is still unknown. Studies of effects of fine-fraction mine tailings waste are limited and the long-term impacts are unknown but necessary to guide best practice management of future mine tailings disposal.

TU107

Toxicity of SPION in the aquatic environment

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Magnetic nanoparticles for biomedical applications are usually formed by a mineral core of a magnetic element, such as iron, nickel, cobalt and their oxides, and an organic coating, such as dextran, polyethyleneglycol, poly (vinylpyrrolidone), streptavidin, poly-L-lysine, polyethylene imide, among others [1]. Superparamagnetic iron oxide nanoparticles (SPION) [2]. For this reason the study of their interaction with the environment is necessary. Microalgae and cyanobacteria are a fundamental link in the food chain because they are primary producers so deleterious effects on their growth will determine a change in the rest of the food chain. We have used two species: *Anabaena* sp (cyanobacterium) and *Chlamydomonas reinhardtii* (microalga). In this study, three different SPION (R42DMSA, R78DMSA and G74) have been characterized. In order to know the stability of the SPION in the different growth media, ζ-potential and aggregation by dynamic light scattering (DLS) were measured. TEM images were taken. The toxicity of each suspension was measured by determining the luminescence inhibition (metabolic toxicity endpoint) of the recombinant bioluminescent cyanobacterium *Anabaena* CPB4337 [3]. Different parameters have been measured in *Chlamydomonas reinhardtii* like growth inhibition, chlorophyll fluorescence, membrane damage, photosynthetic rate, oxidative stress, genotoxic damage and cell viability using different dyes by flow cytometry. Cell magnetization was measured to study the possible internalization of the SPION into the cells. Furthermore, due to their color, SPION could be causing a shading effect, reducing the amount of light reaching the microalgae; to verify this effect, we are studying the effect of physical separation of microalgae cells and SPION suspensions. All SPION tended to form large aggregates in cultures media. The three SPION were toxic for both organisms, but R42DMSA and G74 were more toxic than R78DMSA. **References** Huang J, Zhong X, Wang L, Yang L, Mao H. Theranostics 2012;2:86-102. Prina-Mello A, Crosbie-Staunton K, Salas G, del Puerto-Morales M and Volkow Y. IEEE TRANSACTIONS ON MAGNETICS, 2013 :49:1 Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, García-Calvo E, Santiago S and Rosal R.. Toxicological sciences. 2011 119(1), p. 135–145
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TU108

Toxicity of a novel anti-corrosion nanomaterial towards marine species

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Corrosion is one of the major unsolved problems worldwide. In order to control this problem, corrosion inhibitors (CIs), such as 2-mercaptobenzothiazole (MBT), can be directly added into the protective anticorrosion coatings. Meanwhile, encapsulation/immobilization of active compounds were recently suggested as a very efficient way to minimize the spontaneous leaching of CIs from the coating matrix in the early stages even when corrosion does not exist. In fact, the encapsulation process increases the coating lifetime and reduces the quantity of CIs that are released into the surrounding area due to the capacity to control their leaching rate. Recently, MBT was successfully intercalated in Zn-Al layered double hydroxides, (LDH) for active corrosion protection of metallic structures of the aeronautic, automative and maritime industries. This gave an interesting opportunity to understand the effects in the marine ecosystem. Therefore, the present study aims to assess the hazard that this novel anticorrosion nanomaterial (LDH–MBT) and its major components (LDH–NO_3; MBT) may pose to the marine ecosystem, using 15 marine model species representative of all major ecological trophic levels. Exposure tests of MBT, unloaded nanomaterial (LDH–NO_3) and LDH–MBT were carried out using bacteria (*Vibrio fischeri*), cyanobacteria (*Arthrospira maxima*), microalgae *(Isochrysis galbana, Nannochloropsis gaditana, Phaeodactylum tricornutum, Tetraselmis chuii*), rotifers (*Brachionus plicatilis*), bivalves (*Cerastoderma edule, Mytilus edulis, Ruditapes phillipinarum*), polychaetes (*Hediste diversicolor*), crustaceans (*Acartia tonsa, Artemia salina, Palemon varians*), and echinoderms (*Paracentrotus lividus*). The exposure concentrations ranged between 0.01 and 100 mg/L for most species, including also a control of artificial saltwater. Several sub-lethal and lethal endpoints were measured and L/E/IC_{50} values calculated using nonlinear regression models. These results were transposed to classify the toxicity of each compound according to EC Directive 93/67/EECscheme, adapted by Blaise et al. (2008) for engineered nanomaterials. LDH–NO_3 was not/low toxic 12 out 15 species. MBT was extremely toxic for four species (L/E/IC_{50}< 0.1 mg/L). LDH–MBT was at least 2-fold less toxic than MBT in all cases. Therefore, LDH–MBT seems to be a promising anticorrosion material with low environmental hazard and risk.

TU109

Hazard assessment of innovative anti-fouling nano-based solutions to marine species

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Biofouling is an ecological succession of fouling communities in submerged surfaces with extensive ecological, environmental and economic impacts worldwide. In order to minimize this problem, biocides with anti-fouling properties are commonly used in protective coatings of submerged structures. Some decades ago, organotin compounds were used as effective anti-fouling agents, however they were completely banned in 2008 due to the toxic and biomagnification effects (Arai et al. 2009). As a consequence, a new generation of biocides were developed with lower toxicity and persistence in the environment when compared to organotin compounds. Recently, silica mesoporous nanocapsules were used to encapsulate the booster biocide DCOIT (SiNC-DCOIT) in order to prevent the interaction of biocides with coatings ingredients and control their leaching rate during the early lifetime of conventional paints with environmental and economic benefits. The present study aimed to assess the toxicity of a modified version of this novel engineered nanomaterial, SiNC-DCOIT coated with silver, including now two biocides, to marine species and compare its toxicity with the free counterparts (empty SiNC, DCOIT and $AgNO_3$). Ecotoxicity tests were carried out with eleven marine species, including bacteria (*Vibrio fischeri*), microalgae (*Isochrysis galbana, Nannochloropsis gaditana, Phaedactylum tricornutum*), rotifers (*Brachionus plicatilis*), bivalves (*Cerastoderma edule, Mytilus edulis*), polychaetes (*Hediste diversicolor*), crustaceans (*Artemia salina, Palaemon varians*) and echinoderms (*Paracentrotus lividus*), following standard tests (with some adaptions). Lethal and sublethal endpoints were used. Values of L/IC_{50} for SiNC-DCOIT-Ag (0.014–5.414 mg/L) were higher than the estimated values for both biocides (particularly DCOIT), except for the marine bacteria and diatoms. Bacteria and diatoms are two target groups of organisms involved in the early fouling stages, proving the efficacy of the product. In addition, the decrease in toxicity to non-target species highlights SiNC-DCOIT-Ag as a promising antifouling solution.

TU110

Hazard assessment of an antifouling biocide encapsulated in silica mesoporous nanocapsules

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Biofouling corresponds to the settlement, growth and accumulation of different fouler organisms on submerged surfaces during time. This phenomenon has ecological, environmental and economic key impacts, namely: (a) increase of frictional drag, fuel consumption and CO_2 emissions on vessels; (b) interference on the normal buoyancy of floating devices; (c) reduction of the durability of submerged structures, and (d) contribution for the dispersion of invasive/alien species through ballast water or ships' hulls around the world. Booster biocides with antifouling properties and low persistence in the environment, such as DCOIT (commercially known as Sea-Nine^{TM}), are widely used in state-of-art protective coatings of metallic submerged structures. Recently, the encapsulation of biocides in smart engineered nanomaterials (e.g. silica mesoporous nanocapsules-SiNC) was proposed as an innovative safer-by-design strategy, particularly to prevent and control the excessive release of biocides that occurs in the early stages of the antifouling coating lifetime, which insures an increase of both paint lifetime efficacy and ecosystem protection. The main goal of the present study was to assess the toxicity of the novel antifouling nanomaterial SiNC-DCOIT using model species from the marine environment. Ecotoxicity tests were carried out using a total of eleven species, including bacteria, microalgae, rotifers, bivalves, polychaetes, crustaceans and echinoderms, following standard tests (with adaptions). Several lethal and sub-lethal parameters were used for the hazard assessment. The novel antifouling nanomaterial was always less toxic than the biocide ($L/E/IC_{50}$=0.016–38.20 mg/L and 0.012–1.956 mg/L, respectively). Regarding the fouler species, both compounds were extremely toxic to diatoms, although causing low lethality in mussels. While DCOIT were extremely toxic to 8 out of 9 non-target species, SiNC-DCOIT was only very toxic to 3 out of 9 species. In conclusion, SiNC-DCOIT can be a promising solution with high antifouling efficacy and lower toxicity for non-target species than the commercial biocide.

TU111

TiO2 NPs as "Trojan horse" in the bioaccumulation of personal care compounds in the clam Ruditapes philippinarum.

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In recent years, the use of personal care products (PCPs) has increased notably. This increase together with growing of human population in coastal areas has provoked a constant entrance of emerging pollutants into the aquatic environments. However, data about bioaccumulation and toxicity of PCPs in aquatic biota are scarce. In this research, the clam *Ruditapes philippinarum* has been considered as model organism. The aim of this work was to investigate the role of TiO_2 nanoparticles in bioaccumulation of organic PCPs and the toxic responses of single and mixture exposure experiments using a battery of antioxidant enzymes (superoxide Oxidase, catalase, total gluthathione peroxidase, Se-dependent glutathione peroxidase and glutathione reductase) , lipid peroxidation and DNA damage. In the experiment, four treatments, controls and ethanol controls were run in parallel. Two of the four treatments were single treatments, one of the treatment was 100 ppb of TiO_2 in form of nanoparticles < 100 nm (TiO_2 NPs) and the other one TiO_2 in bulk form. The other two treatments were mixture treatment: a) 100 ppb of TiO_2 NPs plus 10 ppb of a mix of organic PCPs and b) 100 ppb of TiO_2 bulk plus 10 ppb of mix of organic PCPs. The organic PCPs selected were triclosan (antimicrobial), OTNE (fragrance) and benzophenone-3 and octocrylene (UV filters).. Bioaccumulation of inorganic compound such as TiO_2 was almost immediate reaching steady stationary over exposure time; and depuration rate was low. Respect to organic compounds in mixtures, triclosan and benzophenone-3 accumulations were mediated by TiO_2 and was driven by the size of the particles. TiO_2 NPs seems to play a key role in the uptake of organic compounds ("a Trojan horse"), probably due to their high surface area, large pore volume and the presence of high affinity surface hydroxyl groups [1], plus their capacity to stabilize the occurrence of these organic compounds in the cell tissues. NPs provide potential route for the delivery of nanoparticle-associated toxic pollutants to sites where they would not normally reach them. Many NPs, including TiO_2 NPs, have shown a tendency to adsorb other pollutants by binding transitional metal and organic pollutants; in addition they have showed the ability to internalize them into the body and cells. The joint action of the organic compound mix and any of the two forms of TiO_2 provoked significant ($p < 0.01$) changes in the activity of antioxidant enzymes compared (EROD, SOD, CAT, T-GPx, Se-GPx, GST and GR). Despite of higher bioaccumulation of triclosan and benzophenone-3 in the TiO_2 NPs mixture than TiO_2 bulk mixture treatments, toxicity observed could be consequence of compound interactions instead of total bioaccumulation processes. **Keywords:** PCPs, bioaccumulation, trojan horse, oxidative stress. This research has been funded by Junta de Andalucía (PE2011-RNM-7812 project). 1. Moore, M. N., Do nanoparticles present ecotoxicological risks for the health of the aquatic environment? *Environment International* **2006,** *32*, (8), 967-976.

Engineered nanomaterial effects on soil and terrestrial communities (P)

TU112

Oxidative potential of nanomaterials in the eluate of unsaturated quartz sand and natural soils?

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Nanomaterials (NM) are commonly used in many everyday life products and in the end NM will enter the environmental compartments. Soils for example were identified as one important sink for NM with the potential of accumulation of biopersistent NM and/or passage into the groundwater. During this processes they will undergo certain environmental transformation processes which potentially affect the hazard potential of the NM. Based on toxicological results one promising metric describing of the potential hazard of NM is the reactivity or oxidative potential (OP). Within this study we primarily investigated, if it is somehow feasible to apply the spin probe based electron paramagnetic resonance spectroscopy for OP detection in quartz sand eluate after NM treatment and, second, if NM (when passing the soil column) change their OP. Therefore, within the DENANA project in a first approach we performed soil column experiments with a silver and a copper NM in unsaturated quartz sand and detected the OP in the eluate at three different timepoints. At a first time we additionally analysed three types of highly diluted natural soils to their OP by EPR to investigate whether a soil induced OP background can be differentiated to a NM elicit OP. First results indicate that the spin probe based OP analysis by EPR for eluate is feasible. However, the reactivity of the NM seems not to change after passing quartz sand. Furthermore, the OP backgrounds of the highly diluted reference soils differ but seem to be too high for a distinction of NM and/or soil elicit OP. The results have to be confirmed in further experiments and will be presented at the conference.

TU114

Toxicokinetics of zinc-oxide nanoparticles and zinc ions in the earthworm Eisenia andrei

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The toxicokinetics of zinc in the earthworm *Eisenia andrei* was investigated after exposure for 21 days to ionic zinc ($ZnCl_2$) or zinc oxide nanoparticles (ZnO-NP) in Lufa 2.2 soil, followed by 21 days elimination in clean soil. Two concentrations

were tested for both $ZnCl_2$ (250 and 500 µg Zn g^{-1}) and ZnO-NP (500 and 1000 µg Zn g^{-1}), corresponding with EC_{25} and EC_{50} for effects on reproduction. Based on the measured internal Zn concentrations in the earthworms over time of exposure, the kinetics parameters, k_a – assimilation rate constant (g $_{soil}$ g^{-1} $_{body\ weight}$ day^{-1}) and k_e – elimination rate constant (day^{-1}), were estimated using a one-compartment model for either the total Zn concentrations in the soil or porewater Zn concentrations. In the $ZnCl_2$ treatments k_a was higher for total Zn concentrations in soil and in the ZnO-NP treatments for porewater Zn concentrations. The k_e did not differ between the two Zn forms ($ZnCl_2$ vs. ZnO-NP) for either EC_{50} or EC_{25} when related to total Zn concentrations in soil, but at the EC_{50} the k_e related to porewater Zn concentrations was significantly higher for $ZnCl_2$ than for ZnO-NP. It is concluded that differences in kinetic parameters between treatments were connected with exposure concentrations rather than the form of Zn. Zinc was efficiently regulated by the earthworms in all treatments: a 2-fold increase in exposure concentration resulted in less than 2-fold increase in the internal concentration, and after transfer to uncontaminated soil the internal Zn concentrations in the earthworms returned to ca. 111 µg g^{-1} dw in all treatments.

TU115

Bioaccumulation patterns of copper-based pesticides in soil organisms: comparing nano- and non-nano agrochemicals

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Meeting the world's food demands without jeopardizing the equilibrium of ecosystems is one of the grand challenges of modern agriculture. Nanopesticides have been suggested to increase agriculture efficiency, productivity and sustainability, making them attractive for widespread application. Nonetheless, increasing evidences suggest that the processes governing the bioavailability, fate and effects of nanopesticides in soil ecosystems may differ from conventional products due to their high reactivity and uncharacterized nature. This opens new questions about the risks posed by these products to natural compartments, that need to be tackled for developing consistent science-based regulation. The present work was performed in the context of the project NanoFarm (ERA NET SIINN) and focused on developing a valid approach to estimate nanopesticides' potential to bioaccumulate in plants and animals from agroecosystems. Copper-based products were selected as case-study for their worldwide importance in crop protection and for the variety of commercial formulations available, including well-established nano-enhanced compounds. Bioaccumulation experiments were performed using environmentally-relevant concentrations of Kocide®3000 (nano-enhanced copper pesticide formulation), Champion® (non-nano commercial formulation with copper hydroxide) and Copper Hydroxide (active ingredient only). Experiments consisted of a 21-day accumulation phase followed by a 21-day depuration phase using isopods and mealworms. Soil and organisms were periodically sampled and analysed for Cu-content with inductively coupled plasma mass spectrometry (ICP-MS). Kinetic models were used to calculate Bioaccumulation Factor (BAF). Bioaccumulation potential was compared between copper-based compounds and discussed regarding Cu bioavailability in porewater and within the context of current regulatory frameworks.

TU116

A biodynamic understanding of dietborne and waterborne uptake of Ag from AgNO3 and Ag ENPs in a sediment-dwelling oligochaete

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Metal-containing engineered nanoparticles (Me-ENPs) are used in a wide range of consumer products, such as inks, plastics, lubricants, electronics and bioactive coatings. These particles may ultimately end up in the aquatic environment where they can be taken up by a variety of species, from both water and sediment. Yet, a mechanistic understanding of the underlying mechanisms controlling the bioaccumulation of these particles, especially from sediment, is lacking. Here we characterized the bioaccumulation kinetics of Ag after waterborne and dietborne exposures to Ag ENPs in the sediment dwelling oligochaete, *Tubifex tubifex*. Specifically, we exposed worms to a range of environmentally realistic Ag concentrations from Ag added either as Ag ENPs or $AgNO_3$. Worms were exposed in OECD-media (ISO 6341-1892) for 4h to estimate the unidirectional uptake rate constant from water (k_{uw}). Short exposures (5-8h) to sediment amended with Ag ENPs or $AgNO_3$ allowed estimating Ag assimilation efficiency from food, which was used as a proxy to evaluate bioavailability. Ag elimination after aqueous exposure to both forms of Ag was characterized after transferring pre-exposed worms to clean OECD-media for up to 20 days. Results show that *T. tubifex* efficiently accumulated Ag from $AgNO_3$ in solution with an uptake rate constant of 8.2 Lg^{-1} d^{-1}; 25-fold higher than that for Ag ENPs (k_{uw} = 0.34 Lg^{-1} d^{-1}). Once accumulated in tissues after exposure to either Ag forms, Ag was efficiently retained as no significant loss of Ag was detected (p=0.57). However, a high mortality (~50%) was observed for worms on day 17, indicating toxic effects of $AgNO_3$ in these worms. No mortality was observed for worms pre-exposed to aqueous Ag ENPs. Sediment exposures to both Ag-forms showed a low accumulation, with uptake rate constants of 0.002 g g^{-1} d^{-1} for $AgNO_3$ and 0.005 g g^{-1} d^{-1} for Ag ENPs. Silver from both forms was poorly bioavailable (AE ranged from 3-12% for $AgNO_3$ and 0.1-0.5% for Ag ENPs). Ingestion rates (IR) were significantly higher for worms exposed to Ag ENPs compared to $AgNO_3$ ($p< 0.05$), suggesting that the lower bioavailability of the Ag ENPs mitigates the adverse effects of Ag on behavior.

TU117

Toxicity of ZnO nanoparticles in earthworm Eisenia andrei in two agricultural soils with different pH: lethal and reproduction, oxidative stress and coelomocytes assays

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ZnO nanoparticles (ZnO-NPs) are being applied in agricultural formulations, taking advantage of their properties as UV blockers or as fertilizers. It is expected that their application will increase in the future. Despite of the advantages of the use of NPs their release to the environment could impair the different soil living organisms. Therefore, much attention is being paid to the potential risks arising from the use of these materials in agriculture. NPs may interact with the environmental matrices and so their bioavailability and toxicity could be modified. Experiments performed with natural soils are important for the study of NPs toxicity because these more realistically reflect environmental conditions on the availability and hence their effects. However, studies of toxicity of NPs in natural soils are scarce because most of them have been carried out in artificial soils. In this study, the toxicity of ZnO-NPs to earthworms (*Eisenia andrei*) was assessed and compared to bulk ZnO in two agricultural soils with different pH (an acidic soil pH 5.4 and a calcareous soil pH 8.3). The soils were spiked with 20, 250, 500 and 1000 mg Zn kg^{-1} soil. After 28 days of exposure, Zn bioaccumulation in earthworms was measured. Lethal and sub-lethal effects (weight and reproduction) were evaluated. Several endpoints related to the antioxidant defense system (MDA, CAT activity, GST activity and AChE activity) were determined. The coelomocytes were collected to analyze their viability (cell protein content, MTT assay, NRU assay, and ROS assay) and the coelomocytes DNA damage (micronucleus test). The results showed that ZnO-NPs were more toxic when they were applied in the acidic soil. The mortality rate increased and the weight decreased in the earthworms exposed in this soil. Effects on reproduction cannot be assessed in this soil because there was no cocoon production in the control. In the calcareous soil no lethal or sub-lethal effects (reproduction, body weight and biomarkers) were observed in earthworms. Coelomocyte assays were more sensitive than enzymatic assays. In the coelomocyte assay, ROS levels increased at the highest Zn concentration in all treatments. These results could be related to the increase of Zn bioavailability at acidic pH. Differences between ZnO-NPs and bulk ZnO were not observed with some exceptions. This study was funded by the Spanish project: RTA2013-00091-C02-01/02

TU118

Effects of freshly applied and aged silver nanoparticles on selected soil organisms

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Silver nanoparticles (AgNP) may enter the environment through various pathways and may have adverse effects on organisms in soils. The long-term effects of AgNP on soil invertebrates are sparsely investigated as of today. For investigating the effects of AgNP and silver nitrate (AgNO 3) as a reference on the soil invertebrate species *Eisenia fetida*, *Folsomia candida and Hypoaspis aculeifer*, LC_{50}, $EC_{50(Reproduktion)}$ and NOEC/LOEC for both mortality and reproduction were determined. Tests were performed according to the respective OECD guidelines (OECD 222, 226 und 232). With the aim to investigate the influence of the organic substance in soil on the toxicity of the tested substances *Eisenia fetida* was tested with artificial soil using different peat contents (5% and 10%). Testing was repeated after aging aliquotes of the prepared artificial soils three months under the same conditions. Freshly applied AgNP were found to have lesser adverse effects on the test organisms than $AgNO_3$ in three of four test setups. *Eisenia fetida* was found to be the species most sensitive to both compounds. A lower content of organic matter in the artificial soil with 5% peat led to an increase in the effects of AgNP (and $AgNO_3$) on the reproduction of *Eisenia fetida* no matter if freshly treated or aged artificial soil. Ageing of AgNP in the treated artificial soil for a period of three months did not change the effects on reproduction of *Eisenia fetida*, *Folsomia candida* and *Hypoaspis aculeifer* significantly. For $AgNO_3$, a significant and greater decrease than for AgNP in reproductive toxicity was found for all species.

TU119

Effects of pristine and fragmented iron oxide nanomaterials on soil microorganisms - different testing approaches

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Iron oxide nanomaterials (Fe_2O_3-NM) are widely used e.g. for remediation, water treatment as well as in inks, contrast agents, dietary supplements and toner

(http://www.nanopartikel.info/en). Due to this Fe_2O_3-NM will inevitably enter sewage treatment plants (STP) and adsorb to the sewage sludge. Within the European countries sewage sludge is used as a fertilizer in agriculture and therefore the Fe_2O_3-NM reach the terrestrial environment. Our goal was to investigate the effect of two pristine (spherical and rod shaped) and one fragmented Fe_2O_3-NM. For this purpose the study was divided in two steps. In step one, the effects of pristine and fragmented iron oxide nanomaterials on ammonia oxidizing bacteria (ISO 15685) at test start and after 7 and 28 days were determined. In step two an environmental relevant approach was used and sewage sludge was spiked with the Fe_2O_3-NM via a simulation of a STP. Afterwards the sewage sludge was (I) dewatered and (II) treated by anaerobic digestion for 30 days. Next, the sludge was added to a loamy soil in accordance with the German sewage sludge ordinance (5 t dry matter sludge per ha in three years). Long term effects on ammonia oxidizing bacteria (ISO 15685) after 28, 60, 100, and 140 days were investigated. Nominal iron concentrations of 10 and 1000 mg/kg dry matter soil in the first step and 3.4 and 8.5 mg/kg dry matter soil in the second step (applied via sewage sludge) were used. A control without sludge and one with non-spiked sludge served as reference. In the first step no effect at test start was found for the tested products. After 7 days the ammonia oxidizing bacteria were inhibited by 11 and 19% due to the spherical and 4 and 43% by the rod shaped iron oxide nanomaterial. No effect due to the fragmented product was observed. After 28 days there was no significant difference to the control at the different treatments. In the second step no effects on the ammonia oxidizing bacteria could be observed in the long term test after 28 and 60 days incubation in soil at both treatments, dewatered and after anaerobic digestion, respectively. Results for day 100 and 140 are not yet available. The results on the investigated materials so far indicate (a) that the pristine nanomaterials have a short term rather than a long term effect, (b) fragmented products induce no toxic effect on soil microorganisms and (c) no toxic effect if the materials are tested under environmental relevant conditions.

TU120

Multigenerational Exposure of the nematode, C. elegans, to Silver Nanoparticles- Will it Change the Toxic Response?

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Due to their wide range of applications, silver nanoparticles (AgNPs) have become one of the most widely used nanomaterial in industry and consumer products. Adverse effects of Ag are widely known, with effects ranging from oxidative stress, DNA damage and genetic changes, and inhibition of physiological properties, such as growth and reproduction. AgNP toxicity has been reported in a wide range of aquatic and terrestrial species. Most studies, however, only cover a limited timescale of the organism's life stage, rather than the whole lifespan, or even across generation. Therefore, there is little knowledge on effects resulting from multigenerational, chronic exposures. The nematodes, *C. elegans* has a short life span, high fecundity and short generation time, which makes it a perfect model organism for ecotoxicological studies, in particular in multigenerational exposures. The current study was conducted in order to determine whether multigenerational exposure to sub-lethal concentrations of either ionic or nanoparticulate silver (AgNP) could induce alterations in sensitivity to Ag exposure, and a range of other metals (ionic Cu, Cd and Ce, or Ce nanoparticles), and the herbicide paraquat. *C. elegans* were exposed to three sub-lethal concentrations of either ionic Ag or AgNPs. Changes in the response to Ag, either ionic or nanoparticulate, were measured by standard toxicity tests, exposing nematodes to six concentrations of Ag, assessing changes in development, fertility and survival. Nanoparticle characterization was conducted using Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and ICP-MS/OES. This allowed us to obtain a measure of size, stability and properties of the nanoparticles, and total Ag concentrations. Findings so far show a change in resistance to both forms of Ag. Further, slight decreased sensitivity is observed to paraquat, while Ce ions induced an increased sensitivity. No changes were observed for Cu, Cd or Ce nanoparticles. Findings of this study will aid to further improve the understanding of the toxicity of nanoparticles, as well as contribute to our knowledge about the behavior of *C. elegans* in response to toxicants. **Acknowledgements**: Karl Andreas Jensen and Solfrid Lohne. This work was supported by the Norwegian Research Council funded NanoCharm (221391/E40) and NorNanoReg (239199) projects , and the EU NANoREG project grant agreement n° 310584.

TU121

Investigating effects of silver nanoparticles on the soil community - An outdoor TME study

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The production and use of silver nanoparticles (Ag-NP) have markedly increased in the recent years resulting in an increasing release of Ag-NP into the environment. The main entry of Ag-NP into the environment occurs via the use of sewage sludge on arable land making the soil the compartment receiving the highest load of Ag-NP compared to the aquatic environment. Nevertheless, so far, most studies investigating the potential effects of Ag-NP have focused on the aquatic environment, while investigations of effects on soil organisms are scarce. The few studies comprising soil organisms have been conducted with single species exposed to Ag-NP for a short-term period. However, there is a lack of knowledge as to how a natural complex soil community is affected by Ag-NP over a longer period of time. Ecotoxicological impacts on soil communities can be studied using Terrestrial Model Ecosystems (TME). This higher tier approach has been developed to investigate the impact of pesticides on the soil fauna under outdoor conditions over a time period of about one year. It has proven itself as a valid method to detect both effects on the community level as well as on the population level and to observe a potential recovery during the study period. In our project, funded by the German Federal Ministry of Education and Research (BMBF), we have been using the TME approach to investigate potential long-term effects of two differently stabilized Ag-NPs (AgPURE, PVP coated AgNP) on the soil community in comparison to $AgNO_3$. Here we are focussing on effects on collembolans and oribatid mites, two important mesofauna groups, as well as on the community of earthworms. The study addresses the long-term effects of environmentally relevant concentrations. In this presentation first results of the TME study are shown.

Experimental approaches and field studies to investigate ecosystem integrity under multiple stress (P)

TU122

Investigation of soil health assessment system and its application on field sites

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Soil quality assessment is one of the important process to determine the sustainable land use management, and many methods have been suggested such as soil quality index methods and models. Soil quality indicators are used to evalaute sensitivity of changes in soil functions, and these values were applied to indirect approach for intergrated evaluation of soil quality. In the present study, we developed the Korean Soil-health Management and Assessment System (KoSMAS) as the process used to derive a Soil Health Index (SHI) for soil quality evaluation. KoSMAS include a five-step framework: 1) indicator classification, 2) data collection, 3) indicator scoring, 4) indicator ranking and weighting, and 5) caluclation of SHI. SHI are separately derived for four groups of assessment factor under KoSMAS: Soil Productivity (SP), Soil Stability (SS), Biodiversity (Bd), and Safety (Sa). Each assessment factor have various indicator reflecting their characterization, and they were scored and weighted by a regular statisitc method. SHI of each assessment factor was calculated using combination for multification of each score and weights, and they have a scale of 0 to 1, to allow for quatification of soil quality. To verify whether the KoSMAS is well operated on field sites, farm soils including metal contamianted- (FC), remediated- (FR), amended- sites (FA) were constructed. The soil samples were collected at two time points (first and second), and soil-health indicators were analyzed and applied KoSMAS process. Three types of typical crops were planted on filed sites, and the growth and metal bioaccumulation was analyzed for determination of soil productivity and safety. We found the decreasing of SHI on second samples compared with first sample, and these results meant that some of soil properties were changed on field sites. On field test, the crops showed no survival on FA similar with estimation using KoSMAS. The comprehensive quatification of soil quaility is very useful technique for soil management and reuse determination, and the method devleopment should be carefully determined and modified in future studies. *This work was supported by the Korea Ministry of Environment as the GAIA Project (2014000560001). This study was also funded by the Korea Ministry of Environment (MOE) as "The Chemical Accident Prevention Technology Development Project"*

TU123

A semi-field study to evaluate the effects of sulfoxaflor on honey bee (Apis mellifera L.) in cucumber

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Sulfoxaflor is a systemic insecticide which acts as an insect neurotoxin and is used against sap-feeding insects as a sulfoximine, which is a sub-group of insecticides that act asnicotinic acetylcholine receptor (nAChR) competitive modulators. Sulfoxaflor binds to nAChRs in place of acetylcholine. Sulfoxaflor binding causes uncontrolled nerve impulses resulting in muscle tremors followed by paralysis and death. In order to assess the effect Sulfoxaflor on honeybees, a semi-field study in cucumber treated with 22% sulfoxaflor SC was conducted in Hengxi town, Jiangning District of Nanjing under the guidance of EPPO 170(4) (2010). 22% sulfoxaflor SC was diluted in water and applied with a spray application volume of 60L water/mu to protect cucumber at full bloom, at the following rates: 75g a.i./ha (2 applications at 6-day interval) and 100g a.i./ha (2 applications at 6-day interval). Colonies were introduced into the tunnels one day after the first application in the evening (one colony per tunnel), then mortality and behavior of the test bees were observed and recorded every day, meanwhile, samples of flowers were taken and analyzed for sulfoxaflor. The second application was conducted 6 days after the first one, after that, observation of mortality and behavior was conducted for 5 days, and then the colonies were removed from the tunnels (in the evening) and transported back to the apiary. After being back to the apiary, additional observation was conducted for 14 days. Besides, general conditions were assessed before and after exposure, at the end of the field study, and the following endpoints were assessed: weight of the colonies, proportion of bees at each developmental stage, condition of the queens, storage of pollen and nectar. The study is valid since the preliminary assessment of the colonies indicated that the test colonies were in a suitable condition to fulfil the purpose of the experiment (adequately fed, healthy and queen-right colonies, with at least 10000 adult bees, 3 full combs with all brood stage.). Under this experiment condition, sulfoxaflor had no longer lethal effect on bees, and also did not pose any adverse effect on the strength and other conditions of the test colonies.

TU124

Arthropods in off-crop meadows in Northern and Southern Europe: More comparisons of communities and responses to insecticides

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In earlier work we analysed NTA field studies performed with the same active substance but in different cropping systems and in different regions, to provide a first insight into the importance of geographical gradient for the response of non-target arthropod communities to insecticide exposure (1). Here we present an extended meta-analysis that includes studies with other active substances and locations, but restricted to the most vulnerable habitat type (off-crop meadows, 2). Additional analytical methods were used to compare arthropod communities and their responses to insecticides.(e.g. difference in MDD's, PRC on variances). Finally, the availability and suitability of focal species or "indicator groups" (3) for use in terrestrial arthropod community risk assessment of pesticides is discussed. (1) Aldershof, S.A., Bakker F.M. (2012). Comparison of arthropod community responses to an insecticidal active in different geographic regions. In: Alix, A., et al. (2012). ESCORT 3: Linking testing and risk assessment with protection goals. Proceedings of the European Standard Characteristics Of non-target arthropod Regulatory Testing workshop held in Egmond aan Zee, 8-11 March 2010. SETAC press. (2) De Lange, H.J., Larhr, J., Brouwer, J.H.D., Faber, J.H. (2012). Review of available evidence regarding the vulnerability of off-crop non- target arthropod communities in comparison to in-crop non-target arthropod communities. External report EFSA-Q-2011-00791. http://www.efsa.europa.eu/en/supporting/pub/348e.htm. (3) Scientific Opinion addressing the state of the science on risk assessment of plant protection products for non-target arthropods. EFSA Journal 2015;13(2):3996.

TU125

Shell alterations in Lottia subrugosa as putative biomarker for multi-impacted coastal areas

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Lottia subrugosa is a limpet gastropod specie, presenting low mobility and broad geographic distribution along South American coastal zones. In addition, this specie is easily identified and caught in rock shore substrates on which are usually abundant, even under high pollution levels. Previous studies had already detected shells malformations in limpets from sites under different pollution levels. Thus, in the present study the relationship among shell shape parameters and classical biomarkers used in marine pollution monitoring were investigated. At least 100 adult individuals (9.0 to 15.5 mm) of *L. subrugosa* were manually caught in rock shore substrates from 3 sampled sites under different levels of environmental contamination in Santos harbor area (São Paulo state - Brazil). After sampling, the soft parts were removed and analyzed for response of DNA *strand breaks* and lipid peroxidation. The shells were weighed, measured (height, width, length and thickness) and photographed for 2D morphometric geometric analyses. In addition, shells specific gravity was individually obtained by pycnometry. Highly significant differences (Kruskal-Wallis, $p < 0.001$) were observed among the studied sites for all biometric and morphometric parameters analyzed. The specific gravity decreased significantly from the most contaminated areas towards the points under lowest contamination indexes. On the other hand, progressive variations in weight, height and thickness of size normalized shells were detected along the studied gradient. These parameters were positively correlated with DNA damage ($r = 0.78$ and $p< 0.05$) and lipid peroxidation ($r = 0.72$ and $p< 0.05$) responses, which are traditionally used in monitoring of marine contamination. The use of shell alterations has recently been suggested as a putative biomarker for evaluation of multi-impacted areas in Argentina. Therefore, the alterations detected in a small-scale (approximately 5 km) during the present study suggests that these easily measurable parameters can be used as biomarkers of coastal contamination. However, additional studies involving traditionally biomarkers and other multi-impacted coastal areas should be performed to validate the use of this new potential tool. Finally, the wide distribution and abundance of *L. subrugosa* in rock shore substrates, enable this organism as a potential bioindicator for areas impacted by hazardous chemicals.

TU126

Effects of pulses of fungicides and herbicides on the food web structure of a benthic community and the fitness of the benthic omnivore Gammarus roeselii in a microcosm experiment

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In agricultural landscapes, pesticides often occur as mixtures in aquatic ecosystems. Lower concentrations of herbicides and fungicides than the respective effective concentrations can affect non-target organisms through indirect effects while direct effects occur at higher concentration levels. In this study, effects of field-relevant pesticide mixtures on benthic food webs and on the fitness of the benthic omnivore *Gammarus roeselii* were investigated. A 1200 L indoor pond microcosm experiment was conducted to investigate the effects of the three fungicides tebuconazole, pyrimethanil, kresoxim methyl and the three herbicides metazachlor, isoproturon and terbuthylazine on a benthic invertebrate community. The used pesticides are authorised in Germany, frequently found in German surface waters and are supposed to act as model mixtures. Nine microcosms were used to perform a regression design microcosm experiment consisting of controls (n=3) and six different treatments of increasing pesticide concentrations. Concentrations ranged between the regulatory acceptable concentrations and up to the threefold values of the no observed effect concentrations based on chronic *Daphnia magna* experiments, respectively. We expected indirect effects at lower concentrations and direct effects at higher concentrations. Benthic invertebrate communities, sampled from an uncontaminated north-eastern German reference stream, were introduced into the microcosms. One pulse of fungicides was applied at the start of the experiment and was followed by one pulse of herbicides 35 days later. Food web alterations were evaluated using $\delta^{15}N$ and $\delta^{13}C$ stable isotope analyses. Moreover, the fitness of the omnivorous species *G. roeselii* was examined by quantifying its RNA-DNA ratio and glycogen content of single individuals to assess short-term effects. To evaluate long-term effects, additionally, storage lipid content of *G. roeselii* was determined. Furthermore, nutrient concentrations in the water phase were measured biweekly. First results show a shift in the diet composition of *G. roeselii* and a reduction of the lipid content of *G. roeselii* in the pesticide-treated systems, especially at higher concentration levels.

TU127

Effects from pulses of fungicide and herbicide mixtures on the benthic community and emergence pattern of merolimnic insects in a microcosm experiment

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The application of herbicides, fungicides and insecticides in agriculture is the main cause of the intake of pesticide mixtures in surface waters. Low concentrations of herbicides and fungicides can lead to indirect effects like changes of the biotic or abiotic environment or changing competition, whereas higher concentrations additionally have direct effects on non-target organisms. However, risk assessment of pesticides mostly examines the effect of only one pesticide on specific test organisms. Because pesticide mixtures can have different adverse effects than singular pesticides and additionally cumulative effects, it is of high interest to take such mixtures into account in the risk assessment. To examine these effects on non-target organisms such as aquatic macroinvertebrates, an indoor microcosm study with the model system "slowly flowing ditch" was carried out. Benthic invertebrate communities from a pristine stream in north-eastern Germany were introduced in the microcosms. Six pesticides, which are admitted in Germany and have repeatedly been detected in surface waters, have been chosen as model mixtures. First, a pulse of fungicides consisting of tebuconazole, pyrimethanil and kresoxim-methyl was applied, followed by an herbicide pulse, 35 days later, consisting of metazachlor, isoproturon and terbuthylazine. The pesticide mixtures were applied with increasing concentrations in six microcosms, with the lowest concentration being the RAC (regulatory acceptable concentration) and the highest being about three times the NOEC (no observed effect concentration; *Daphnia magna*, chron.) for each pesticide. With this range of concentrations a shift from indirect to direct effects was simulated. Three additional microcosms were used as control systems. Endpoints were the emergence pattern of merolimnic insects and effects on the benthic community. The results show a shift in the benthic community, mainly caused by *Gammarus roeselii*, *Gammarus pulex* and *Baetis vernus*, in the microcosms with the two highest pesticide concentrations, where those taxa were strongly reduced. Similar to the alteration of the benthic community there was a reduction of the emergence of *B. vernus* in the microcosms with the two highest concentrations.

TU128

Individual and combined effects of drought and the fungicide fluazinam on aquatic ecosystem structure and functioning.

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Worldwide, freshwater ecosystems face a variety of threats due to human activities such as pollution, over-exploitation and reduction of natural habitats by water abstraction. Pressure on these ecosystems is expected to be even further aggravated by climate change with a likely increase in drought events. Therefore, it is important to understand the combined effects of drought and toxicants on aquatic ecosystems. In this study we investigated the individual and combined effects of drought (achieved through a decrease in water level for 10 days) and pesticide stress (100 µg/L of the fungicide fluazinam) on aquatic ecosystem structure and functioning. We performed three cosm experiments, one performed indoor and one outdoor using a sequential timing of the stress factors, while the third experiment was performed indoor using a coinciding timing of the stress factors. All treatments were applied to 5 replicates resulting in a total of 20 systems. Endpoints measured in time were fluazinam concentration, physico-chemical parameters, zooplankton, macro-invertebrates, chlorophyll-a and decomposition. The results of the experiments are currently under evaluation and their preliminary results will be presented. Preliminary results show that generally the drought treatment resulted in a temporal increase of zooplankton and chlorophyll-a, while copepods were negatively affected by the fluazinam treatment and the cladocera increased in abundance. In the experiments using a sequential timing of the stress factors hardly any interactive effects, other than additive, of the two stress factors were found. Only in the indoor experiment a synergistic effect was found in the form of a delayed recovery of nauplii in the combined treatment compared to the separate treatments. In the experiment using a coinciding timing of the stress factors more synergistic interactive effects were found, indicating the importance of the timing of stress events on their interactive effects. These findings stress the need for deeper insight into how multiple stressors can affect the structure and functioning of aquatic ecosystems and associated services in order to improve the ecological risk assessment of stressors.

TU129
Advanced sewage treatment as a kingly gift for aquatic ecosystems?
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Wastewater treatment plants (WWTPs) are known as major sources for the emission of micropollutants as, e.g., pharmaceuticals, pesticides, household chemicals, or ingredients of cosmetics and human care products. In order to cope with this problem, new technologies in waste water treatment that are based, e.g., on an additional powdered carbon stage or on ozonation in combination with granular carbon or sand filtration have been developed during the last decade. Up to now, however, little is known about the advantages of these additional cleaning steps for ecosystem health. The BMBF-funded project SchussenAktiv*plus* has examined the efficiency of several wastewater treatment technologies for (1) the reduction of micropollutants, (2) the related reduction of adverse effects analyzed by biotests and biomarker studies as well as (3) the elimination of (resistant) microbes. With respect to ecological advantages of WWTP upgrading we focused on a large WWTP connected to the Schussen River, a major tributary of Lake Constance, which has been upgraded by a powdered activated carbon stage by the end of 2013. Here, we investigated micropollutants in effluents, surface water, sediment, and biota, and studied effects in fish and invertebrates at different biological levels prior to and after the upgrading. In addition, a large series of biotests was applied in order to link exposure to effect data by means of mode-of actions based results. The nearby located Argen River, also a tributary of Lake Constance, served as a reference river. In summary, we could show that all investigated technologies reduced, micropollutants significantly, but also substance-specifically, by more than 80%.The additional powdered activated carbon stage at the large WWTP, in addition, resulted in lower concentrations of micropollutants in the surface water and in biota downstream this WWTP. Plausibly correlated to this, fish and invertebrates were found to be more healthy after the WWTP upgrading and showed less genotoxic effects, tissues of higher structural integrity, less detoxification activity, and a better development. Estrogenic effects were of minor importance in the Schussen River. From a socio-economic view, the public costs of potential WWTP upgrading in the entire catchment area of the Schussen were calculated.

Insights and challenges concerning the bioavailability of organic chemicals and communication implementation in risk assessment (P)

TU130
Passive dosing of toluene increased the accessibility of PAHs and heterocyclic PAHs in industrially contaminated soil
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Polycyclic Aromatic Hydrocarbons (PAHs) are hydrophobic contaminants with a strong tendency to sorb to soil constituents. For risk assessment and also for possible remedial actions of such contaminants, it is crucial to understand the binding mechanism to specific soil constituents and to quantify the accessible fraction of the individual contaminants. In this study, *Sorptive Bioaccessibility Extractions* (SBE) with a large capacity absorption sink (polydimethylsiloxane, PDMS silicone) were applied to quantify the accessible PAH fraction in industrially contaminated soil. Passive dosing was then applied to determine the effect of the competitive sorbate toluene on the release and bioaccessibility of PAHs. Finally, the *contaminant trap* was applied to generate industrially contaminated soil dominated by desorption resistant PAHs, which then was tested for bioaccessibility in the absence and presence of the competitive sorbate. SBE experiments revealed an accessible PAH fraction of 41 ± 1% (∑16 US EPA PAHs + 5 further heterocyclic and methylated PAHs) for the industrially contaminated soil. The addition of toluene below its saturation level (by passive dosing) revealed competitive binding and resulted in an average increase of the accessible fraction to 49 ± 2%. The competitive sorption of toluene enhanced predominantly the accessibility of higher molecular weight PAHs (log $K_{ow} > 6$). Competitive binding was verified using the same soil, accommodating only desorption resistant PAHs as prepared by incubation in contaminant traps. Passive dosing of toluene resulted here in a release of 13 ± 1% of initially in-accessible PAHs. Increased PAH desorption upon addition of toluene indicates, that adsorption to high affinity sites contributes significantly to PAH retention in the industrially contaminated soil investigated in this study. These findings highlight the importance of co-pollutants at contaminated sites, which deserve specific consideration as they may increase accessibility and as a consequence exposure of PAHs.

TU131
Roles of DOM quality in bioavailability-oriented bioremediation strategies with PAHs
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Biodegradation of PAHs can be enhanced through bioavailability-oriented strategies, such as solubilization (what affects phase exchange-processes), tactically- driven microbial mobilization, and attachment to interfaces, what allows the direct acquisition of the soil/sediment-associated contaminant. This overview presentation will focus on the recent research efforts in our group, where we have specifically addressed the influences of DOM quality on these bioavailability-related phenomena. We used, for our studies, different experimental models to assess bioavailability, and these included Tenax extraction, dual 14C/residue analysis of microcosm samples, dynamic passive dosing with PDMS, biphasic NAPL/water systems, and column systems. Different model DOM sources, of dissimilar quality, were used, and included humic acids, root exudates, biosurfactants and organic fertilizers. We found that biodegradation of poorly bioavailable PAHs was enhanced by (bio)surfactants (Environ. Sci. Technol. 48:10869-10877, 2014), the targeted fertilization of free-oil phases or NAPLs (Environ. Sci. Technol. 45:1074-1081, 2011), by modulating the deposition and tactic motility of microbial degraders in porous media (Environ. Sci. Technol. 46:6790-6797, 2012), and by root exudates (Soil Biol. Biochem. 57:830-840, 2013; Environ. Sci. Technol. 49:4498–4505, 2015). However, a negative influence on biodegradation of PAHs by humic acids (Environ. Pollut. 184:435-442, 2014) and biosurfactants (Environ. Pollut. 205:378-384, 2015) was found if they prevented cell attachment to the PAH-loaded PDMS sources. These findings have led us to design innovative remediation protocols that include the sequential actions of different DOM sources that specifically operate on the fast- and slow-desorbing PAH fractions. These influences of DOM quality on bioavailability are relevant not only for innovation efforts in bioremediation but they have also connections with the determination of bioavailability of organic chemicals in retrospective and prospective risk assessment and regulation (Environ. Sci. Technol. 49:10255-10264, 2015).

TU132
Characteristics of PAH tar oil contaminated soils - Black particles, resins and implications for treatment strategies
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Tar oil contaminations are a major environmental concern due to health impacts of polycyclic aromatic hydrocarbons (PAH) and the difficulty of reaching acceptable remediation end-points. Six tar oil-contaminated soils with different industrial histories were compared to investigate contamination characteristics by black particles. We provide a simple method tested on 6 soils to visualize and identify

large amounts of black particles (BP) as either solid aggregates of resinified and weathered tar oil or various wood/coke/coal-like materials derived from the contamination history. These materials contain 2 to 10 times higher PAH concentration than the average soil and accumulated 42 to 86% of PAH in the sand fraction. The PAH contamination in the different granulometric fractions was directly proportional to the respective total organic carbon content, since the PAH were associated to the carbonaceous particulate materials. Significantly lower (bio)availability of PAH associated to these carbonaceous phases is widely recognized, thus limiting the efficiency of remediation techniques. In addition, we developed a conceptual model of the limited mass transfer of PAH from resinated tar oil phases to the water phase and emphasize the options to physically separate BP based on their lower bulk density and slower settling velocity.

TU133

Enhanced vermiremediation of hydrocarbon contaminated lands with biosurfactants.

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Polyaromatic hydrocarbons (PAH) contamination in soil continues to be one of the biggest environmental challenges because of the persistence and carcinogenicities of the contaminants. There is a need in seeking environmental friendly ways to remediate these pollutants and vermiremediation provides a promising solution Earthworms because of their burrowing activity give way to the accumulation of several lipophilic organic pollutants in their surrounding environment via the absorption of the contaminant through their body wall and also through intestinal uptake which occurs when soil passes through the gut. This make them suitable organisms for the remediation of PAH's from contaminated lands. Biosurfactant produced by bacteria is known to be able to enhance bioavailability of hydrocarbons; this research investigates the effect of biosurfactant on vermiremediation using *Lumbricus terrestris & Eisenia hortensis*. We conducted a 3 month experiment by exposing these two species of earthworms to phenanthrene (Phe) and fluoranthene (Flu) in spiked soil samples 60 and 120 $mgkg^{-1}$. We established a time response relationship between the concentration of Flu and Phen and length of exposure, with 85% degradation within 7 days of soils enhanced with biosurfactant and only 40% remediation of soils without biosurfactant in both species. Results indicate that vermiremediation enhanced with biosurfactant is a promising and fast technique to remediate PAH contaminants

TU134

The INSSICCA research project: INnovative Strategies to establish Safe livestock rearing systems In Chlordecone Contaminated Areas

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The chlorinated polycyclic ketone pesticide chlordecone (CLD) was used from 1971 until 1993 in the French West Indies to fight against the banana black weevil (*Cosmopolites sordidus*). Its application resulted in a long term pollution of soils which is thought to last 5 to 7 centuries for the heaviest polluted soils. About 1/4 of the total agricultural acreage of the two French overseas departments (Guadeloupe, Martinique) are moderately to heavily polluted and national survey plans carried out since 2008 in slaughterhouses revealed unexpected contamination of animal products. A research program involving a broad research network is currently on going. It aims at evaluating local animal rearing systems in terms of livestock exposure to CLD, at characterizing the bioavailability of CLD, its behavior and metabolization in the animal organism, and at establishing strategies to bind CLD via activated carbon in the digestive tract. On the basis of the results obtained, safe local livestock rearing systems will be proposed in agricultural areas historically contaminated by CLD, and assessed in terms of economical efficiency and social acceptability. Preliminary results showed that, in tropical conditions, ingestion of soil is far from being negligible but it can be modulated by the forage offer in order to reduce livestock exposure to CLD. Regarding the potential to bind CLD in order to limit its absorption, the most promising results are obtained when activated carbon is added and maturated into contaminated soil. Where such approaches cannot be applied, there is a remaining potential to decontaminate the animals by applying a depuration period: CLD half lives in the organism are currently established. Such results are of great importance and will allow in a near future to establish innovative safe local livestock rearing systems in agricultural areas historically contaminated by CLD. The approaches implemented in this project and the proposed innovative strategies to secure the rearing systems have also to be considered as a "model approach" to be applied further in organic pollutant contaminated areas worldwide.

TU135

Comparison of the efficiency of in situ soil amendment and alimentary supplementation by activated carbons to limit Chlordecone transfer to piglets

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Chlordecone (Kepone) (CLD) is a highly persistent pesticide formerly used in French West Indies. Nowadays high levels of this pesticide are still found in soils and represent a subsequent source of contamination for outdoor-reared animals. In that context, sequestering matrices such as activated carbons (ACs) are believed to efficiently decrease the bioavailability of such compounds. The present study intends to test the respective efficiency of two sequestering strategies using ACs in the animal production context: (i) ACs supplemented to feed or (ii) ACs as a soil amendment. This study involved 20 piglets randomly distributed into 5 experimental groups (4 replicates). All of them were exposed through one artificial soil to 10 μg of CLD per kg of BW. This soil was amended by 2% (mass basis) of either ORBO and DARCO, the two ACs tested, and given to 2 experimental groups. Two other groups were given additionally a feed containing 0.5% (mass basis) of one of the ACs. Only the experimental groups exposed through amended soil with one of the two ACs presented a significant decrease of concentrations of CLD in liver and adipose tissue in comparison with the control group ($p< 0.001$). This decrease was particularly high for a coconut shell activated carbon (ORBO) where relative bioavailability was found to be lower than 1.8% for both tissues. Overall, AC introduced in CLD contaminated soil has thye potential to strongly reduce CLD bioavailability whereas sequestration of CLD during digestive processes via supplemented feed was not efficient.

TU136

Toxicokinetic of chlordecone in small ruminants: linearity and half-life

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The former use of chlordecone (CLD) in the French West Indies has resulted in long-term pollution of soils, with 10% of the agricultural soils considered as heavily contaminated (> 1 $mg.kg^{-1}$ DM). CLD is known to be potentially transferred into animal products of livestock reared outdoors, mainly through involuntary soil ingestion. Indeed, several studies indicate that soil bound CLD ingested is fully available in the intestinal lumen and absorbed at a rate of 100%. The objective of this study was to characterize CLD toxicokinetic in the caprine species and in the ovine species, more specifically: 1- the CLD half-life in organism 2- the linearity of the toxicokinetic as a function of the doses. Indeed, the fact to know the evolution of the toxicokinetic in response of increasing doses appears essential to further extrapolate the results obtained for different exposure levels, i.e. in field situation. Three groups of 5 ewes and a group of 5 goats received an intravenous single dose of CLD (0.04, 0.2 or 1 mg/Kg body weight for the ewes and 1mg/kg body weight for the goats). Plasma samples were taken by venipuncture at defined times up to 84 (ewes) or 160 (goats) days post-dosing and stored during 24 hours at 4 °C before centrifugation at 1500 x g for 10 min to get serum samples which were stored at - 20 °C before analysis by high-resolution gas chromatography. The first results in goat show a half-life of CLD in serum of 16.9 (± 1.5) days. The further analysis should be highlight a half-life of the same order of magnitude in ewes. This value shows a possible strategy of decontamination due to the short half-life of CLD, obtained in small female ruminants that did not excrete fat matter. In addition, a comparison of the area under the plasma concentration-time curves (AUC) should highlight the linearity of the toxicokinetic for this scales of the doses. This linearity would allow the extrapolation of the half-life of the CLD in small ruminants in field situation, when the concentration in tissue is above the Maximal Residue Limit.

TU137

Current-use pesticides: their fate in soil from the perspective of the bioavailability approach

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Pesticides used in agriculture represent one of the largest inputs of chemicals to soil. Nowadays, risk assessment associated with the presence of a chemical in soil is based on the total concentration although evidence has been collected that total soil concentration does not properly reflects the environmental risks as it does not allow the factor of bioavailability/bioaccessibility to be considered. In this study, the total concentrations of pesticides in soil with their bioaccessible concentrations assessed by three non-exhaustive extraction techniques using sorbents (namely XAD, silicon rubber and Empore disk) operating under infinite sink conditions were provided. After the optimization of the extraction time and sorbent amount, soils either with natural occurring residues or spiked to desired concentrations were exposed to the above mentioned sorbents and data on the total amounts and bioaccessible fractions compared over a range of pesticides (including non-polar, polar and ionizable current-use pesticides) and soils with varying physico-chemical properties.

TU138

Fate (sorption/desorption and bioavailability) of DDE in sterile and non-sterile soils

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Microorganisms play an important role in the bioavailability and fate of organic pollutants in soil when influencing their dissipation from soil as well as sorption/desorption equilibrium by continuous degradation and/or production of surfactants etc. Microorganisms are of a vital importance for soil quality and an evitable factor, and, therefore, soil microbiology can be considered as one of the key aspects characterizing a particular soil environment. To distinguish between the abiotic and biotic factors in the fate of organic pollutants, comparative studies performed at both sterile and non-sterile conditions are required. Similarly, in order to study interactions of pollutants with other soil biota, such as bioaccumulation in plants and soil macrofauna, soil sterilization may be required to rule out the confounding effects of soil microorganisms. However, several studies have reported that sterilization can affect chemical and physical properties of soils, including soil organic matter (SOM) chemistry. Since SOM is the principle sorbing domain for organic pollutants, changes induced by soil sterilization itself may potentially alter bioavailability of pollutants. Our study investigated the impact of soil sterilization by ?-irradiation and of soil spiking with organic solvents on SOM characteristics and bioavailability of DDE as a model soil contaminant. Two soils were employed in our study, a field contaminated soil where sterilization followed contamination and a spiked soil where sterilization preceded contamination. The changes in SOM chemistry induced by sterilization process and spiking were assessed via the diffuse reflectance infrared Fourier transform (DRIFT) technique. Two different chemical methods, XAD extraction and solid phase micro-extraction, SPME) and the earthworm bioaccumulation were used to asses DDE-bioavailability before and after sterilization. The results of the sterile and non-sterile variants were compared between soils and treatments in order to provide a better insight in the role of microorganisms and sterilization on pollutants´ fate (sorption, desorption, bioavailability) in soils.

TU139

Balancing toxicity mitigation and bioaccessibility in sludge biodegradation studies with cationic surfactants.

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Cationic surfactants are important ingredients in various household products, personal care products and industrial processes. High removal rates in waste water streams lower the potential impact of these chemicals on environments receiving waste water effluent and sludge, and microbial biodegradability in sewage treatment systems is thus a critical environmental fate parameter. The biodegradability is typically assessed with standardized OECD assays with activated sludge, e.g. through measurement of mineralization to CO_2 in ready biodegradation tests, e.g. the headspace test (OECD 310). However, due to CO_2 detection limits, test concentrations in the assay are normally~10-20 mg C/L, at which several cationic surfactants are toxic or have inhibitory effects on the inoculum. Using test concentrations below toxic effects would require radiolabeled material, which is costly. In this study, we evaluated whether addition of two different inert and inorganic sorbents (silicon dioxide and illite clay) could mitigate the toxicity of the common antiseptic ingredient cetylpyridinium chloride (CPC). Furthermore, we compared these OECD 310 results with similar sorbent addition studies in the OECD 301f biodegradation assay, which may be a more cost-effective way of measuring biodegradability using manometer measurements of air pressure decrease due to oxygen depletion.

TU140

Application of passive sampling measurements for determining compliance with EU WFD environmental quality standards

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Anthropogenic contaminants have been found in surface waters at concentration levels that cause concerns regarding potential impacts on ecosystems. Regular water quality monitoring should be carried out to satisfy the requirements of EU Water Framework Directive (WFD, 2000/60/EC). The current monitoring system relies on the spot sampling, followed by extraction and analysis in the laboratory. Spot sampling is well established and validated, but limitations such as only providing a snapshot of the water quality status which is not always representative have often been discussed. Here, passive sampling is recognized as an alternative method, which has the merits simple sampler construction and application, determination of time-weighted average concentration and *in situ* sampling. Although passive sampling measures the bioavailable fractions of contaminants, which is advantageous in ecotoxicity studies, this does not satisfy environmental quality standards (EQS) criteria as defined in the WFD since these are based on the total aqueous concentration (2013/39/EC). Therefore, in this study, we assess the possibilities and reliability of passive sampler measurements to support routine WFD monitoring programs. The interpretation of passive sampling measurements for comparison with EQS was explored. Two types of passive samplers, Polar Organic Chemical Integrative Sampler (POCIS) as a kinetic sampler and Mixed Polymer Sampler (MPS) as an equilibrium sampler, were deployed in the river Saar and a wastewater treatment plant in Germany over five weeks (May-June, 2015). As a result, 27 out of 46 target compounds were detected, including 8 priority substances listed in the WFD. The identified dissolved concentration of contaminants were then converted into total concentrations for comparison to the EQS by considering the chemical speciation and hydrophobicity (i.e., log K_{OW}) of each compound. This study contributes to enhancing the capability and reliability of passive sampling technique so that it might be accepted for regulatory purposes.

Antibiotics and Antibiotic Resistance in the Environment: Ecological Fate and Effects, Resistance Development and Implications for Human Health (P)

TU141

Hospital effluents, not an exclusive source of emerging contaminant spread in sub-saharian urban rivers

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The contamination of freshwater resources with anthropogenic pollutant is a growing concern of interest because safe and readily available is a need for human. The situation is particularly alarming in developing countries where most rivers and lakes are receiving urban and hospital wastewater without any prior treatment. Furthermore, hospital effluents are known to play a major role in the emergence and the spread of antibiotic resistance genes (ARGs) because they discharge a high rate of antibiotic resistant bacteria (ARBs) in a highly selective environment. Cumulative with the knowledge gaps in antibiotic prescription and the free access to last resort antibiotics, under-developing countries may represent a broth culture to antibiotic resistance emergence, maintenance and dissemination. In this study, we investigate the contamination of sediment and the resistance profile of extending-spectra β-lactamase *E. coli* (ESBLECs) isolated from 4 urban river subjected to hospital outlet pipes (HOP) discharge in order to determine the effect of HOP discharge on urban river quality and the pattern of antibiotic resistance dissemination. ARGs (bla_{TEM}, $bla_{CTX\text{-}M}$, bla_{SHV} and *aadA)* and selected bacterial species (i.e *E.coli*, *Enterococcus*, Pseudomonas *spp.*) where quantified in sediments using quantitative PCR (qPCR), toxic metals content were quantified by ICP-MS, and ESBLECs isolates where subjected to pulse-field electrophoresis to assess their clonality and characterized for their resistance to metal and β-lactams resistance. The results highlight the great concentration of toxic metals in sediments (47.87 (Cr), 204 (Cu), 1077 (Zn), 2.07 (Cd), 124.40 (Pb) and 3.94 (Hg), in mg.kg^{-1} at the HOP reject point), and the high content of FIB and ARGs copy number in all sampling sites including control site and downstream the HOP discharge indicating that hospital effluents are not an exclusive source of the biological contaminant entering the urban rivers. The analysis of ESBLECs profile showed the high diversity of clones disseminated in rivers (150 isolates, 69 pulsotypes), a global resistance to Zn, Cu, Ni and Pb and a high resistance profile against β-lactams (100% resistant to CFM, CEC, ATM, AMP, FEP, CXM, CTX, PRL; 60% to SAM and 37% intermediate to MEM). These findings indicates the human and environmental potential risk link to exposure to these contaminants and the need of developing strategies to limit the spread of these emerging contaminant.

TU142

Fluconazole resistant Candida albicans from environmental water and associated clinically relevant efflux pump genes

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Introduction *Candida albicans*, a major fungal pathogen of immune-compromised people were isolated from surface water of the North West Province, South Africa. The life threatening systemic infections which these species could cause, are treated with antifungals such as fluconazole. The latter is used in the HIV-AIDS treatment regime as prophylactic treatment against *Cryptococcus* and *Candida* infection. Recently we have demonstrated that environmentally isolated *Candida albicans* was resistant to several antifungal agents especially fluconazole. It has been suggested that efflux pumps coded for by *CDR1*, *FLU1* and *MDR1* genes could be responsible for the observed resistance phenotypes. The aim of this study was thus to determine antifungal if/which of these efflux genes potentially mediated resistant mechanisms of these environmental *Candida albicans* isolates. Yeasts isolated by a membrane filtration technique, were purified and identified using 26S rRNA gene sequencing. Kirby Bauer disk diffusion method was used to determine antifungal susceptibility. End-point PCR was used to detect the presence of efflux mediated genes. The fragments were sequenced and subjected to BLAST and subsequent phylogenetic analysis. Thirty seven *Candida albicans* were identified from 5 selected rivers; Mooi River (46.9%; 17 isolates), Harts River (18.9 %; 7 isolates), Marico River (13.5 %; 5 isolates), Schoonspruit River (13.5 %; 5 isolates) and Crocodile River (8.1 %; 3 isolates). All the isolates were completely resistant to azoles. Efflux pump genes were detected in most of the isolated species, *CDR1* (85%), *FLU1* (85%) and *MDR1* (70%),respectively. The sequences of these genes were compared to available sequences from clinical isolates. Phylogenetic analysis showed high sequence similarity between environmental isolates and clinical isolates. These yeast species were found in environmental water that is used for household and religious purposes that include direct exposure of individuals. Resistance to the azoles and the detection of efflux pump genes is cause for concern which renders these antifungal agents ineffective against disease states caused by the yeasts. This will be a major problem particularly for the immune-compromised sector of the community of the North West Province. This sector accounts for 15% of the population of this province. Results call for further research into the links between fluconazole use, levels in the environment and selection for antifungal resistance.

TU143

Dissemination and spread of antibiotic resistance genes in benthic microbial biofilm communities in rivers: a mesocosm experiment

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Antibiotics are used extensively in both human and veterinary medicine as well as in agriculture and are continuously introduced into the aquatic environment. The dissemination, persistence and spread of antibiotics, resistant microbes and resistance genes with potential adverse impacts on public health have become an emerging environmental problem. A variety of studies reported the occurrence of antibiotic resistance genes (ARGs) in bacteria as well as the occurrence of antibiotics in wastewater and surface water. However, the question of how bacterial communities evolve and respond to this sub-inhibitory antibiotic stress in river ecosystems has rarely been addressed. For this reason, mesocosm experiments are used in this project to expose microbial biofilm communities taken from river sediment to systematic scenarios of antibiotic stress. Sub-inhibitory levels of erythromycin, roxythromycin, ciprofloxacin, tetracycline and penicillin over extended periods of time are introduced in the experimental setup. This allows to investigate the response of antibiotic resistance to the antibiotic selection pressure in terms of changes in the abundance of resistance genes. Moreover, the microbial biofilm communities inside the mesocosms are exposed to a specific size fraction of particulate matter from municipal wastewater to simulate the impact of human/animal-associated bacteria after discharge of treated wastewater into urban river systems. Specific ARGs are studied within whole bacterial communities after DNA extraction by using quantitative PCR methods. Shifts in the community composition are monitored by denaturing gradient gel electrophoresis (DGGE) and DNA sequencing. Analytic determination of antibiotic concentrations will be performed using LC-MS/MS.

TU144

Determining the minimal selective concentrations of macrolides in complex microbial communities

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Antibiotic resistance is now a global health crisis, it is predicted that by 2050 that antimicrobial resistance will overtake cancer as the leading cause of death, with over 10 million deaths per year. The environment is thought to be an underappreciated reservoir of resistance. As well as environmental microbes producing antibiotics for competitive means, continual release of antibiotic from human sources can lead to a build-up of antibiotics in the environment. Only recently has the environment been considered as an area where selection for antibiotic resistance occurs. Concentrations of antibiotics in the environment are significantly lower than concentrations used in the clinic to treat infections and lower than MIC. Data published in 2011 and 2014 by Gullberg *et al.* showed that selection can occur at low antibiotic concentrations, representative of those found in the environment, using single species competition assays. In 2015, macrolide antibiotics were added to the European Commission's Water Framework Directive, as they have frequently been found at high concentrations in the environment and there is growing concern about their impact.\n \n We therefore investigated the selective potential of macrolide antibiotics in aquatic systems using complex microbial communities. To do this, a week-long evolution experiment was set up using environmental complex community samples. This meant that determining how antibiotics affect a population, rather than a single species, can be investigated. Real-time PCR was used to determine prevalence of resistance genes with in the community and how they change over time and with different concentrations of antibiotics. Our data suggests that current environmental concentrations of macrolides do not have a selective effect on bacteria in experimental assays.

TU145

In vivo exposure of marine mussels to Sulfamethoxazole: uptake, bioconcentration and metabolization

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Sulfamethoxazole is a sulfonamide antibiotic extensively used in human and veterinary medicine due to its bactericidal broad spectrum and low cost. It's presence in coastal waters, and particularly estuaries, harbors and lagoons have been widely studied with levels ranging from low ng/L up to less than ten μg/L [1]. The presence of this compound in the aquatic environment may result in a chronic exposure of marine organisms, especially in the case of filter feeding organisms due to its polar nature which makes it directly bioavailable. Besides, there is a growing concern due the possible development of antibiotic resistance in aquatic organisms from aquaculture and the potential risk to human health through the ingestion of this seafood. Therefore, to characterize sulfamethoxazole uptake, bioconcentration and metabolization in mussels become a relevant issue due its high consumption. With this purpose an experiment was undertaken in a marine mesocosm where *Mytilus galloprovincialis* were exposed to an environmental relevant concentration of sulfamethoxazole during 96 h. After this period, the mussels went through a depuration phase of 24 h under real commercial conditions, in the facilities of a shellfish enterprise dedicated to import and export bivalves for human consumption. A control tank free of contaminant was held in the same experimental conditions. Seawater, mussel and haemolymph were sampled at different interval times in order to measure the concentration of compound and its possible metabolites. The sulfamethoxazole concentration in seawater was kept constant at 9.5±1.5 μg/L during the exposure phase. Up to 4 μg/L of sulfamethoxazole were measured in haemolymph and metabolization by mussel was not observed during the experiment. Only 24 h of clearance were long enough to completely remove the antibiotic from the mussels and therefore, assure a safe consumption by population.

Reference 1.Rodríguez-Mozaz, S., Álvarez-Muñoz, D., Barceló, D., *Environmental Problems in Marine Biology: Methodological Aspects and Applications. Chapter: Pharmaceuticals in Marine Environment: Analytical Techniques and Applications.*, ed. G.B. Ariza J.L , T.2017: CRC Press, Taylor & Francis.

TU146

Persistence of the antibiotic sulphametoxazole in river water microcosms

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Many recent studies have reported the ubiquitous occurrence of antibiotics in aquatic ecosystems. Antibiotics can both kill natural microbial populations and act as a pressure for the selection of resistant bacteria. Following administration, antibiotics are only partially metabolized and, therefore, a large amount is excreted unaltered or as active metabolites via urine and faeces; consequently, human antibiotics reach wastewater treatment plants. The latter are not specifically designed for antibiotic removal, and consequently these molecules are released directly into the receiving environments. The fate and effects on health ecosystem of these compounds is not still well known. Sulfamethoxazole (SMX) is one of the most widely synthesized sulfonamides, prescribed to treat urinary infections; it is also used in veterinary practice, aquaculture and livestock breeding. Its mechanism of action is based on the inhibition of folic acid synthesis in bacteria. SMX has been detected in groundwater, in effluents of WWTPs and in surface water, including drinkable water. SMX has been reported to be not readily biodegradable, resistant to hydrolysis and the photo-degradation as a possible degradation process in surface waters. Data on biodegradation in natural water ecosystem are quite scarce. In this context, the aim of the present work was to investigate the persistence of the antibiotic sulphametoxazole in river water, focusing on both the biodegradation and photodegradation processes. For this purpose, two different microcosm experiments were set up using river water treated with 500 μg/L of SMX in the presence or absence (river water sterilized) of the natural microbial community. In order to evaluate SMX biodegradation, the first experiment was performed in the dark. In the second one, river water microcosms were incubated under UV-light, in order to evaluate the possible photodegradation of SMX. At fixed times, water sample were collected from microcosms for measuring SMX residual concentrations over time by-using HLPC-UV. The disappearance time of 50% of the initial SMX concentration (DT_{50}) was evaluated in both experiments. Moreover, the effects of the antibiotic on the natural microbial community were assessed in terms of cell vitality, abundance and phylogenetic diversity comparing the treated river water microcosms with those no treated one.

TU147

Antimicrobials modify decomposer detritivore interactions - the role of substances' target microorganisms

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Leaf-associated microorganisms (i.e., bacteria and fungi) as well as leaf-shredding macroinvertebrates play fundamental roles in the breakdown of leaf litter, a crucial function within detritus-based stream ecosystems. However, antimicrobial substances such as antibiotics and fungicides may negatively affect the latter group of organisms via both waterborne exposure and their diet. The diet-related pathway includes the dietary exposure towards antimicrobials adsorbed to leaf material as well as effects on leaf-associated fungi, on which shredders' nutrition heavily relies (i.e., microbial conditioning). To expand the limited understanding of the importance of these two effect pathways, we examined the effects of the antibiotic ciprofloxacin (CIP) and the fungicide azoxystrobin (AZO) on the amphipod model shredder *Gammarus fossarum*. In separate experiments, gammarids' leaf consumption and physiological fitness were assessed using a 2x2-factorial test design: amphipods were fed leaf material microbially conditioned in the presence or absence of CIP (500 μg/L) or AZO (30 μg/L). At the same time, *G. fossarum* was either cultured in antimicrobial-free medium or was directly exposed towards the respective antimicrobial agent for 24 d. In comparison to the control, gammarids' leaf consumption and growth were increased (~17%, ~54%) when the food was conditioned in the presence of CIP. This is most likely explained by CIP-induced negative effects on leaf-associated bacteria, releasing fungi from competitive pressure, which may have triggered an enhanced food quality. In contrast, no diet-related effects where observed in the AZO experiment, whereas waterborne exposure reduced the shredder's survival (~50%). Moreover, animals surviving until the termination of the experiment showed an impaired leaf consumption (~40%) and feces production (~41%) as well as a negative growth when subjected to waterborne exposure. Thus, we showed that both antibiotics and fungicides can affect shredders' physiology with potential implications in their population dynamics and functioning. Moreover, these substance classes differ strongly regarding their effect direction and the relevance of the assessed effect pathways (i.e., waterborne vs. diet-related). As both substance classes can co-occur in aquatic ecosystems, a sensible next step would be to assess their combined effects to receive a more holistic picture of how antimicrobials affect key players in aquatic ecosystem functioning.

TU148
ASSESSMENT OF PRIORITY ANTIBIOTICS AS A PHARMACEUTICAL POLLUTANTS FOR UKRAINE
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The objective of our study - determination of predicted environmental concentration (PEC) of priority pharmaceuticals pollutants of surface waters in Ukraine among antibiotics. \nIn Ukraine research of pharmaceuticals in surface waters has not been carry out yet. Growing concern over global pharmaceutical pollution causes to study this issue in Ukraine too. As the basic formula for calculation PEC in surface water we used well-known methodology by Besse J.P.\nWe carried out a modification of this formula. In the calculation we used WHO data of health statistics (ATC/DDD system proposed by WHO). The ATC/DDD system is a tool for provision of reliable and comparable data for our understanding of national pharmaceutical markets. Overall consumption of medicines has expressed as defined daily doses (DDD) API per 1 000 inhabitants and per day (DIDs).\nUsing this approach we calculated value of PEC / PNEC for antibiotics that most consumed in Ukraine. Antibiotics amoxicillin, ciprofloxacin and sulfamethoxazole have highest priority as pharmaceutical pollutants representing a risk of negative impact on the environment in Ukraine.

Applying Bioaccumulation Data to Better Inform Human and Ecological Risk Assessment of Chemicals (P)

TU149
Application of trophic magnification factors (TMFs) under the Water Framework Directive: some practical advice on selecting and determining a TMF
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Directive 2013/39/EU amending and updating the Water Framework Directive (2000/60/EC) and its Daughter Directive (the so-called EQS Directive: 2008/105/EC) sets Environmental Quality Standards for biota (EQS_{biota}) for a number of bioaccumulative chemicals which can pose a threat to both aquatic wildlife (piscivorous birds and mammals) and human health via the consumption of contaminated prey or the intake of contaminated food originating from the aquatic environment. Member States (MS) of the European Union will need to establish programs to monitor the concentration of 11 priority substances in biota and assess compliance against these new standards for surface water classification. The biota standards essentially refer to fish and should be applied to the trophic level (TL) at which contaminant concentrations peak, so that the predator of the species at that TL is exposed to the highest contaminant levels in its food. For chemicals that are subject to biomagnification, the peak concentrations are theoretically attained at TL 3 to 4 in freshwater food webs and TL 5 in marine food webs, where the risk of secondary poisoning of top predators should also be considered. An EU-wide guidance effectively addresses the implementation of EQS_{biota} (EC 2014). Flexibility is allowed in the choice of target species used for monitoring because of the diversity of both habitats and aquatic community composition across Europe. According to that guidance, the consistency and comparability of monitoring data across MS should be enhanced by adjusting the data on biota contaminant concentrations to a standard trophic level using the appropriate TMF. In this context, the selection of a TMF value for a given substance is a critical issue, since this field-derived measure of trophic magnification can show an appreciable amount of variability, related to the characteristics of ecosystems, the biology of organisms, the physicochemical properties of contaminants, the experimental design, and statistical methods used for TMF calculation, etc. In this presentation, guidance is given for the selection of TMFs for reliable applications within the context of the WFD (i.e. adjustment of monitoring data and EQS derivation). Based on a series of quality attributes for TMFs, a decision-tree is developed to help end-users select the "most reasonable" TMF.

TU150
Bioaccumulation and Effects of Emerging Contaminants in the Aquatic Organism Brown Trout
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Emerging contaminants have been ubiquitously detected in effluent from wastewater treatment plants (WWTPs) worldwide. Emerging contaminants may bioaccumulate in aquatic organisms such as fish, and subsequently enter the food chain, potentially posing a human health risk. In this study, the impact of contaminants in WWTP effluents with respect to accumulation in the aquatic vertebrate fish was investigated. First, a priority list of emerging contaminants in the aquatic environment was compiled, based on the frequency and level of occurrence as well as their toxic potencies, and included pesticides, pharmaceuticals, biocides, and industrial chemicals. Second, a sensitive and selective method involving quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction and online-solid phase extraction combined with liquid chromatography high resolution tandem mass spectrometry was developed to quantify 61 emerging contaminants in fish tissue. Finally, the method was successfully applied to wild fish samples collected from upstream and downstream of several WWTPs in Switzerland. Apparent bioaccumulation factors were calculated based on the ratio of the internal concentration in fish tissue over that analyzed in water. Furthermore, the bioaccumulation of these emerging contaminants was correlated to the biomarker gene expression level from genes covering all phases of cellular detoxification to get insight into the effects of WWTPs effluent. The findings of this study may have important implications for both the scientific community and policy makers in addressing the safety concerns of the emerging contaminants.

TU151
Derivation and Evaluation of Partitioning Properties for the Bioaccumulation Assessment of CMP Priority Ionogenic Substances
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Environment and Climate Change Canada (ECCC) recently published an approach for classifying the risk of organic chemicals using weight of evidence driven chemical profiling. The *Ecological Risk Classification System* (ERC) prioritizes substances that may be of higher concern from a combination of high potency and high probability of exposure (ECCC 2016, http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=A96E2E98-1). Among the organic chemicals ranked for further assessment using the new ERC approach, it was found that ~50 (unique CAS numbers) with surfactant properties were considered high or moderate priority. Another ~45 chemicals with surfactant properties were considered low priority. Surfactants are challenging chemicals for any level of risk assessment modeling, since most are ionizable organic chemicals (IOCs) and the current suite of risk assessment tools are not developed to deal with the predominant ionic form of such chemicals. Consequently, methods and algorithms to derive logP are not amenable to most types of surfactants. A key element in modeling the bioconcentration factor of ionogenic compounds is to specifically include the water-phospholipid partition coefficient (K_{plipw}) of the ionic

compound. This $K_{plipw,ion}$ can be measured using artificial phospholipid phases with relative ease compare to logP measurements. As a first screening to define if such measurements are experimentally feasible for selected surfactants, we used software that applies quantum-chemical calculations (i.e., COSMOmic, a submodule of COSMOtherm) on the ionic form for the sorption affinity to phospholipid bilayers. The software calculations on several clusters of the selected anionic and cationic surfactants will be evaluated here along with recommendations for further testing.

TU152

Guidance for using solid-phase microextraction in fish bioconcentration studies according to OECD TG 305

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Fish bioconcentration studies needed for the safety assessment of chemicals are commonly performed according to the OECD Test Guideline 305 (OECD TG 305). The studies result in a bioconcentration factor (BCF) calculated by the chemical's concentration in fish divided by its concentration in the water phase. The method most often used for the extraction of the water phase is conventional solvent extraction by liquid–liquid extraction (LLE), which is considered as an exhaustive extraction method yielding total water concentrations. However, conventional water analysis in fish bioconcentration studies can result in an underestimation of BCF values of highly hydrophobic organic chemicals (HOCs) when their freely dissolved water concentrations are reduced by sorption to organic matter but total water concentrations are extracted. However, with solid-phase microextraction (SPME), the discrimination between total and freely dissolved analyte concentrations is possible. Therefore, the 2012 revised OECD TG 305 recommends the use of SPME for the extraction of water concentrations of (highly) HOCs. The use of SPME in fish bioconcentration studies will be explained in a Guidance Document on OECD TG 305, which will be finalized in 2017. The present contribution presents relevant information on SPME as given in the upcoming Guidance Document supported by results from a bioconcentration study with rainbow trout, where three (highly) HOCs were extracted comparatively by LLE and SPME. These results help to explain factors influencing bioconcentration systems and provide relevant insights about the use of different extraction methods in fish bioconcentration studies with highly HOCs carried out according to OECD TG 305.

TU153

Heavy metal contamination of vegetables planted in Lagos soils

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Vegetables are important components of human diet as they are rich sources of vitamins, minerals, fibers, and antioxidants. Heavy metal contamination of vegetables would definitely pose direct or indirect health risks to the humans. The human health concern as a result of consumption of heavy metal contaminated vegetables prompted this study. Twenty one samples of vegetable from seven different types of vegetables namely *Amaranthus viridis, Celosia argentea, Corchorus olitorius, Lactusa sativa, Telfairia occidentalis, Allium tricoccum and Talinum triangulare* were collected from four vegetable farmlands sited at Iyana-Iba at latitude 6.482^{0}N and longitude 3.200^{0}E; Baruwa Pipeline at latitude 6.594^{0}N and longitude 3.272^{0}E; Isheri-Oshun at latitude 6.511^{0}N and longitude 3.292^{0}E; and Idi-Araba at latitude 6.514^{0}N and longitude 3.356^{0}E. Soil samples around the roots of the plants harvested to make a total of twenty one soil samples from the four farmlands. With the use of Atomic Absorption Spectrophtometer (AAS), non essential, toxic elements (heavy metals) which are associated with many chronic diseases in humans were analysed in different commercial vegetables and soils from selected vegetable farmlands in Lagos. These heavy metals include Cadmium, Chromium, Copper, Iron, Lead, Nickel and Zinc. The concentrations of Lead in *Corchorus olitorus*, *Celosia argentea* and *Lactuca sativa* were above the recommended maximum acceptable limits by WHO. The concentrations of Nickel, Iron, Lead, Chromium, and Cadmium in the vegetables were higher than the recommended EU standards. Zn and Cu in all the vegetables of the four farmlands were lower than recommended EU standards. Zinc, Nickel, Copper, Lead and Cadmium were lower than the recommended EU standards for cultivated soils while Iron and Chromium were higher. There were no statistical significant differences in the levels of Ni, Fe, Cu, Pb, Cr and Cd in the vegetable ($p > 0.05$) while Zn ($P = 0.04$) has a significant difference in the vegetables. The heavy metals had no significant difference ($p > 0.05$) in the soils. The risks posed by some of the heavy metals in some of the farmlands are hereby presented.

TU154

In vitro to in vivo extrapolation of hepatic metabolism in fish: update on development of OECD Test Guidelines and Guidance Document

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Chemical biotransformation represents the largest source of uncertainty in chemical bioaccumulation assessments, and model-based estimates of chemical bioconcentration in fish may be greatly improved by including biotransformation rates, as measured in vitro. In vitro substrate depletion assays, using rainbow trout hepatocytes or liver subcellular fractions (S9), have been successfully developed to provide estimates of fish biotransformation. A multi-laboratory ring trial, coordinated by the ILSI Health and Environmental Sciences Institute (HESI), was recently completed. This study involved six laboratories, each of which performed substrate depletion assays on six test chemicals in both systems (rainbow trout liver S9 fractions and rainbow trout cryopreserved hepatocytes) to determine in vitro intrinsic clearance ($CL_{in\ vitro\ int}$). Results successfully demonstrated assay reliability within and across laboratories and similar performance of substrate depletion assays using the two biological systems. For all test chemicals, hepatic clearance values determined by the two test systems were in good agreement (within 2-fold). Based on the successful results of this ring-trial, the two test guidelines (*"Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes"* and *"Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fractions")* have been drafted and are accompanied by by a Guidance Document. The launch of OECD WNT review of these draft documents is planned for 2017. The Guidance Document provides additional information on how to conduct the tests as well as how to apply the measured in vitro biotransformation rates to predict bioconcentration factors (BCFs). This includes guidance on the selection of the assay system (e.g., primary hepatocytes vs. liver S9 fractions), specific considerations for testing chemicals, use of negative and positive controls, BCF extrapolation models, and application of the two test methods to other fields beyond BCF prediction. This poster will provide an overview of the Test Guidelines and Guidance Document, as well as an update on progress and timelines.

TU155

PBDEs and legacy POPs in the Antarctic environment and biota

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In the current study, we measured the concentrations of OCPs, PCBs and PBDEs in blubber samples collected from different sex and age classes of Weddell, Crabeater and Ross seals and three killer whale biopsies. The goals were to (i) determine the concentrations of different POPs in seals and killer whales, also relative to expected health effects; (ii) compare the levels and profiles of POPs in the four investigated species and elucidate the influences of sex, age, species type lipid content and weight on contaminant concentrations and patterns by applying multivariate and univariate statistical techniques; and (iii) determine the bioaccumulation and biomagnification behavior of POPs in the Antarctic food web. Geometric means of OCPs generally ranged from 30 – 92 ng/g lipid, 165 – 182 ng/g lipid, 166 – 230 ng/g lipid in *Lobodon carcinophagus*, *Leptonychotes weddellii* and *Ommatophoca rossii* respectively. In *Orcinus orca*, OCP concentrations (mean 2,112 ng/g lipid) were ~ 1.0 – 2.0 order of magnitude higher than concentrations in the seals. Unlike the legacy pollutants, mean PBDE concentration in *Orcinus orca* was within the range observed for *Lobodon carcinophagus*. Several interesting trends occur across the investigated animals. First, orcas displayed much greater concentrations than the seals, in-line with their trophic position. Second, juvenile seals were close to the adults from the same species with respect to POPs concentrations in blubber. Third, there were no clear trends evident in POPs concentrations across the seal species. Concentrations of HCBz and PBDEs were fairly similar between all 3 seals, mostly within a factor of 2-4. In the case of HCBz, this could reflect the similar exposure of all species to this POP that is closest to an equilibrium distribution in the global environment; for PBDEs this might reflect the ability of all species to metabolize PBDEs taken up. In summary, concentrations of legacy pollutants, dominated by DDTs and chlordanes, were still much higher than PBDEs. In both seals and killer whales, PBDE concentrations were much lower than the legacy pollutants, but within the range observed in previous studies. This in turn may highlight the role played by local sources (research stations and ice melting) in polluting this unique aquatic environment.

TU156

Prediction of in vivo biotransformation in fish and human for the refinement of bioaccumulation

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Biotransformation significantly influences the fate and toxicological profile of chemicals in organisms. Biotransformation rate information is useful for the refinement of bioaccumulation hazard assessment metrics and exposure and risk estimates in humans and fish. For instance, the first-order, whole body, primary biotransformation rate constant (k_B) and corresponding half-life (HL_B) can play an important role in mitigating the bioaccumulation potential of hydrophobic chemicals in fish, i.e. resulting in lower accumulation than what is expected based on LogKow alone. Since the experimental quantification of bioaccumulation related parameters is cost intensive, time consuming, and poses ethical concerns regarding the number of tested animals, the use of *in silico* models based on Quantitative Structure-Activity Relationships (i.e. QSAR) represents possible solutions to support hazard and risk assessment when experimental data are scarce or absent. In this poster, we summarize the current state of the art for the development and application of *in silico* models based on QSARs for the prediction of *in vivo* biotransformation-related parameters, such as HL_B in fish and humans. These linear QSARs are based on different molecular descriptors (i.e. molecular fragments, and global molecular descriptors) and have been developed in compliance with the current requirements for application of QSARs for regulation. Finally, we briefly discuss the complementary role of *in vitro* biotransformation rate estimation and the subsequent *in vitro*-to-*in vivo* extrapolation (IVIVE) process for refining bioaccumulation model predictions. Multiple benefits are derived from the application of these *in silico* approaches, such as the reduction of experimental costs and animal testing, and the improvement of green chemical design/production processes via screening prior to synthesis to avoid undesired properties (e.g. biopersistency and potential for bioaccumulation). The research presented provides recommendations to the scientific and regulatory community to improve the current *in silico* methodologies and to focus future experimental needs for the refinement of existing models.

TU157

Reporting of the relative affinity (M) constant of hemoglobin for carbon monoxide in animal world: an historic, systematic and meta-analysis review.

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Background: The Haldane (M) constant represents the oxygen/carbon monoxide ratio bound to hemoglobin and is a key value of physiological and pathological models exploring carbon monoxide (CO) metabolism or exposure. However, results on (M) values appear to be inconsistent among studies and species studied. A better estimation of (M) value and a species consideration will enforce accuracy of CO studies results and allow elaboration of reliable models. Aims and methods: We performed a systematic review and meta-analysis in order to determine and compare (M) values according to species and experimental conditions. We used electronic libraries (PubMed/Embase/JSTORE) and performed a manual search on references from 1895 to January 2016. For each article, we collected: species, age, temperature, pH, atmospheric pressure, presence of 2,3-DPG rate or methemoglobin, type of hemoglobin. Statistical analyses were performed with JMP®Pro12 software, SAS©, USA. Results: The systematic review found 25 articles addressing (M) constants including 135 individual data and 51 mean values concerning mammalians, laboratory animals, fish, fetal hemoglobin and invertebrates. (M) values at physiological temperature and pH were assessed only for adult (137 [119-153]) and fetal (135 [112-168]) sheep, dogs (215 [193-218]), mice (194 [118-278]) and rats (191 [177-200]). Correlation between (M) values and temperature was confirmed for sheep and horse. The lack of data did not permit to conclude about correlation between (M) and pH. No species presented statistically equivalent (M) values compared to human blood at 209 [190-257]. Conclusion: Our results confirm that (M) values vary according to species. Only the influence of temperature is confirmed. A formal measurement of the (M) constant in the given conditions of experimentation should be performed for each animal model study on carbon monoxide. Further homogeneous studies are necessary to compare (M) values among different animals and to determine the species whose (M) value is most comparable to human blood.

Bioremediation and phytoremediation of contaminated environments (P)

TU158

Rhizoremediation of an historical contaminated site in Brescia (Italy): comparing the current emission of PCBs and PCDD/F to air from soil and other sources

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Among the national priority polluted sites, the SIN Brescia Caffaro is located in a mid size city Brescia, (200,000 inhabitants) in northern Italy. The site derived from the activity of the former Caffaro s.p.a., a chemical factory among the largest former polychlorinated biphenyls (PCBs) producer in Europe, which produced such chemicals for more than 50 years up to mid 80'. About 100 Ha of agricultural areas were contaminated by a mix of Persistent Organic Pollutants (POPs, mostly PCBs, dioxins, furans) and heavy metals (Hg, As) in variable concentrations, often exceeding the safety values. Such concentrations are orders of magnitude higher than the typical background anthropogenic values. Since the city of Brescia is located within an industrial district where current emission and secondary sources of POPs are also present (incinerators, smelters, etc.), this site represents a challenging case to evaluate the prevalence of a source over another and select the type of remediation activities. Concentrations of PCBs (more than 80 congeners) and PCDD/Fs in soil were measured averaging samples taken at surficial and deeper layers in 45 points located in the contaminated agricultural areas (totalling about 100 Ha). Concentrations of PCBs and PCDD/Fs were then used in the SoilPlus model (a multilayered dynamic multimedia fugacity model including a two layer dynamic air compartment) to predict hourly air concentrations of selected congeners and dioxin/furan classes for an entire simulation year. Such concentrations were compared to PCB and PCDD/F air concentrations measured in Brescia in five different areas at different seasonal intervals. Principal component analysis showed that calculated PCB fingerprints in air overlap with the measured ones, with seasonal concentrations varying accordingly (the predicted winter concentrations are closer to the measured), confirming that volatilization from soil determines current concentrations. The situation is different for PCDD/F where only furans seem more correlated with soil emissions, showing that dioxin sources are recent. A comparison of soil PCB fingerprints with Aroclor fingerprints confirms that the present congener mixture is mostly of highly chlorinated congeners, while PCDD/F fingerprints confirm the prevalence of furans.Such information is crucial to evaluate the impact and potential remediation effectiveness of rhizoremediation in presence of old and current sources.

TU159

Eco(toxico)logical effects of biochar in contaminated and non-contaminated soils

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Biochar application to soils is being considered as a means to sequester carbon while concurrently improving soil functions and mitigate risks of soil pollutants. However, there is currently a lack of data concerning the effects of biochar on the ecological function of soils, namely soil fauna. Maximum application doses (MADs) of biochar allowing to be applied to soil without compromising its ecological function has not yet been established. In the present project, both contaminated and non-contaminated (control) soils have been amended with biochar to provide information on the remediation effects of biochar as well as the possible detrimental effects of biochar added to soils at increasing doses spanning up to 20%. Nematodes (Caenorhabditis elegans), a representative of soil micro-fauna, were used as a biological indicator for evaluation of the remediation potential and possible negative side-effects of biochar in soils. Solid-phase microextraction was applied for the determination of porewater concentration of contaminants in polluted soils and for the estimation of exposure concentrations. Porewater concentrations were related to the observed toxic effects at various biochar doses. To further reveal whether biochar negatively influences food availability to nematodes and soil pH, the nematode toxicity test and porewater concentrations were measured at low and high E.coli densities and both natural and pH-adjusted conditions.

TU160

Effects of DDE occurrence on the Solanum lycopersicum rhizosphere under different organic carbon sources

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Contamination by Persistent Organic Pollutants such as DDTs (*p´p´*-DDT and its metabolites *p´p´*-DDE and *p´p´*-DDD) is an important threat to soil ecosystem quality. Plant-based clean up technologies are as sustainable solutions to contaminated soil remediation. They rely on the physiological abilities of selected plants to decrease pollutants from soil through different processes occurring in the rhizosphere involving symbiotic and non-symbiotic interactions with natural microorganisms. *Solanum lycopersicum* is known to be able to accumulate DDTs in roots, owing to exudate production, which makes this contaminant bioavailable in soil. In this context, a greenhouse experiment was performed using pots filled with DDE-contaminated soil and watered with different kinds of dissolved organic carbon (DOC). Control soils (without plant and/or DDE) were also performed. Finally, we obtained 12 different conditions. Soil and plant samples were collected 40 days after the DDE exposure. The effects of the different treatments on the natural microbial community of the rhizosphere were evaluated in terms of microbial abundance, viability, structure, dehydrogenase activity and possible DDE degradation. Moreover, roots and shoots of the tomato plants were also sampled and analysed for the determination of the pesticide bioconcentration. The main results from the different experimental conditions will be discussed.

TU161

Biochar addition improves edaphic conditions and favours root elongation and biomass production of Sarcocornia fruticosa growing in acidic mine wastes

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Phytomanagement consists on the manipulation of soil-plant systems to control environmental trace metal fluxes. This study aimed to assess the effects of biochar addition for the phytomanegement of salt marshes polluted by acidic mine wastes. Experimental columns (15x30 cm) were filled with the acidic mine wastes (pH~4.7), with and without biochar application. Two types of biochar were used (from pruning trees -BPT- and from sewage sludge -BSS-) at a dose of 6% d.w. *Sarcocornia fruticosa* (common halophyte species in Mediterranean salt marshes) seedlings were planted in the experimental columns, which were put inside bigger containers. During the first month the containers were filled with tap water (water level ~5 cm above the column top -the whole column underwater-), while during the second month half of the water was removed from the containers (water level ~15 cm below the column top -only the lower 15 cm of the columns underwater-). This cycle was repeated four times during one year. The pH and redox potential (Eh) were regularly checked and porewater samples extracted for measuring water soluble organic carbon (WSOC) and water soluble metal concentrations (Cd, Mn, Pb and Zn). The extracted pore water was used for the performance of germination experiments with *Sarcocornia* seeds (seed germination and growth of radicle, hypocotyl and cotyledons). Plant total fresh weight and length were measured at the end of the experiment. Biochar BPT increased the soil pH to ~6.0-6.5 while biochar BSS to ~5.5. The presence of vegetation did not have any effect on the soil pH. Both types of biochar decreased the Eh values, which was attributable to the estimulation of the soil microbial activity due to the input of WSOC. Higher pH induced by biochar application led to decreasing porewater metal concentrations, regardless of the presence of vegetation. Both biochars had similar effect on Cd and Pb porewater concentrations, while biochar BPT was more efficient decreasing Zn and Mn concentrations. Seed germination was not affected by the different treatments assayed. However, in the absence of biochar there was not development of the radicle, hypocotyl and cotyledons in the germinated seeds. Plants grown in the treatments without biochar were the smallest (less fresh weight and shorter). In the amended treatments, plants grown with biochar BSS showed the greatest fresh weigth, althought there were no differences in total length compared to the biochar BPT treatments.

TU162

Integrated system combining microalgae and vertical flow constructed wetlands for the treatment of urban wastewaters

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The releases of partially treated or untreated wastewaters into water bodies are, because of the high nutrient loads, the main causes for the eutrophication of the aquatic ecosystems. Facing this reality it is necessary to develop technologies that might recover the quality and minimize the toxicity of the wastewaters before disposal in receiving bodies. Since the conventional wastewater treatment technologies are based on physical and chemical methods which are mostly costly and environmentally unsustainable, there is a need to seek other alternatives. In this sense, the present study investigated the performance of an integrated system, combining the sequential use of microalgae (MA) and vertical flow constructed wetland (VFCW) for the treatment of wastewaters produced at a university campus. General characterization parameters of the raw and treated wastewaters included COD, BOD_5, conductivity, pH, turbidity (NTU), total phosphorus (P), ammonia nitrogen, total Kjeldahl nitrogen (NTK), nitrate, absortiometric color and thermotolerant coliforms. Ecotoxicity and phytotoxicity assays were performed using respectively *Daphnia magna* and *Lactuca sativa*, whereas the genotoxicity of the wastewaters was assessed by using *D. magna* and *Allium cepa*. The results revealed that the major environmental impacts of the studied wastewaters are associated with the high eutrophication potential, due to high COD, BOD_5, $N\text{-}NH_3$ and total P values, pathogenic load and genototoxicity. The results also showed that the integrated system (MA + VFCW) was not able to satisfactory reduce the COD and total P values. Nevertheless, the MA + VFCW system achieved very promising results for the nitrogen removal, with emphasis on $N\text{-}NH_3$ removal. Neither the raw wastewaters nor the treated wastewaters were phytotoxic. The integrated system completely eliminated the ecotoxicity and genotoxicity of the raw wastewater and showed decontamination potential. Therefore we can conclude that the integrated system emerges as an innovative environmental technology and, with minor adjustments might be efficiently used in large-scale and eventually replace conventional wastewater treatment systems, which besides been financially unfeasible are extremely impacting to the natural systems.

TU163

Evaluation of the sulfonamide antibiotic persistence in the presence of the aquatic plant Lemna minor

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Sulfonamide antibiotics are currently prescribed to treat urinary infections in human and veterinary therapeutics. Following administration, they are only partially metabolized and, therefore, a large amount is excreted unaltered or as active metabolites via urine and faeces; in the case of human administration, the antibiotic residues can reach wastewater treatment plants and, through effluents, the receiving waterbodies. Currently, there is little known about the fate and effects on health ecosystem of these compounds. Among Sulfonamides, sulfamethoxazole (SMX) is one of the most detected antibiotics in river waters. SMX disrupts the folate biosynthetic pathway in bacteria, which is identical to that of plants, raising concerns about toxicity effects on non-target organisms. Therefore, improving our knowledge on the persistence of SMX, especially in natural water ecosystems, is particularly needed. In this framework, macrophyte aquatic plants, such as Lemna minor L, growing in wetlands and contaminated water bodies, can be a useful tool to evaluate the interaction between aquatic plants and organic pollutants. Moreover, the plant-natural microbial community interactions on the antibiotics biodegradations have to take into accounts. In this context, the aim of this study was to improve our knowledge on the biodegradability of SMX in river water, focusing on the effect of the antibiotic on both the natural microbial community and plants. Lab-scale experiments were set up by using microcosms containing river water treated with 500 μg/L of SMX both in the presence/absence of the plant L. minor and in presence/absence (sterilized river water) of the natural microbial community. Other microcosms (Controls) were set up with no-treated river water (without the antibiotic). The experimental set was kept in a growth chamber under controlled conditions. Water samples were collected at fixed times and the SMX residual concentrations were measured over time, by a chromatographic method (HPLC-UV). The effects of the antibiotic on the natural microbial community were assessed in terms of cell vitality, abundance and phylogenetic diversity. Moreover, physiological and biochemical determinations were performed on plants. SMX residual concentrations in plants were also analyzed at the end of the experiment; in this case, Accelerated Solvent Extraction (ASE) followed by Solid Phase Extraction (SPE) purification methods were used.

TU164

Effect of rhamnolipid biosurfactant in biodegradation of slow-desorption PAHs in contaminated soils

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The aim of this work was to determine the bioavailability to degrading microorganisms of native PAHs present in contaminated soils, with focus on slow-desorption fractions. We used different concentration of rhamnolipid, a microbially-produced biosurfactanat, a concentration above its critical micelle concentration to enhance the biodegradation of slowly desorbing PAHs. The soil samples exhibited different levels of pollution (a soil originated from a polluted site, and two soils which had been treated by bioremediation). We developed an accelerated biodegradation assay, consisting in the incubation of the samples in the presence of a sufficient number of PAH-degrading microorganisms. The addition of ^{14}C-labelled PAH to the samples allowed the measurement of mineralization (production of $^{14}CO_2$) as a physiological indicator of the biodegradation process. After mineralization reached the plateau, the residual concentrations of the native compounds were determined by extraction and HPLC analysis in replicate samples that contained no ^{14}C and that had been maintained under the same conditions. In addition, we used desorption methods, well represented by Tenax desorption, which allows a permanent HOC aqueous concentration of almost zero, and therefore sorption of the HOC back to the sediment/soil can be neglected. The assessment of the kinetics of PAH desorption, to determine the magnitude of the slowly desorbing fractions present in the polluted soil, seems to be a prerequisite for this optimized surfactant role in bioremediation.This method is widely accepted as a valid tool to assess bioavailability of slowly-desorbing HOCs (Environ. Sci. Technol. 48:10869-10877, 2014; Environ. Sci. Technol. 45:3019-3026, 2011). In this way, we could identify situations involving different degrees of bioavailability: from heavily polluted soils containing highly bioavailable compounds, to soils previously treatedwith bioremediation, showing a reduced bioavailability. Our results do not show a clear difference in the rates of mineralization of 14C-labeled PAHs with and without surfactant; however the measurement of residual concentrations of native PAHs evidenced the promoting effect of the surfactant on the biodegradation of the slowly desorbing fractions. These results have implications for the management of PAH pollution involving bioremediation.

TU165

Assessment of an integrated system for the treatment of domestic wastewaters generated in rural areas: reduction of load parameters and toxicity

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The establishment of appropriate water and wastewater management systems is a vital issue, especially in developing countries. Sanitation data from these countries reveal serious hygiene, health, economic and social implications that highlight the urgent need to develop less expensive, more efficient and easy-to-maintain technologies which may suit regional realities. Thus, the present research investigated the efficiency, applicability, and environmental sustainability of an integrated wastewater treatment system constructed in a property located in a rural area of the town of Vera Cruz, southern Brazil. The system consisted of an anaerobic unit (upflow anaerobic sludge blanket combined with an anaerobic filter), four subsurface flow constructed wetlands, and two photoreactors. *Hymenachne grumosa* was the selected macrophyte. General characterization parameters of the wastewaters included COD, BOD_5, NTK, $N\text{-}NH_4$ and total P analysis. Ecotoxicity assays were performed using *Daphnia magna* whereas the cytotoxic, genotoxic and mutagenic potential of the wastewaters was assessed by using the *Allium cepa* test. Results showed reductions between 93% and 97% for COD and decreases by 97-98% for BOD_5. Additionally, 97% of the TKN, 100% of the $N\text{-}NH_4$, and more than 78% of total P were removed from the wastewater. The system reduced fully eliminated the acute ecotoxicity of the raw wastewaters. Samples of the system's output presented a significant increase of the cell division in relation to the negative control (NC) in the *A. cepa* assays indicating a possible capacity to remove the cytotoxic potential of the wastewaters. Furthermore, the samples collected after the treatment did not present significant differences of chromosome aberrations and of micronucleated cells when compared to the NC, indicating absence of genotoxic and mutagenic potential. The assessment of the integrated system shows that is was efficient for the treatment of domestic wastewaters generated in rural properties. There is a potential for the reuse of the treated wastewaters, however, it is necessary to have a permanent analytical control of the biological indicators, since this parameter might unable the waterÂreuse even for less noble uses, such as sanitary discharges.

TU166

Biochars and compost for in situ remediation of diffusely contaminated sites

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Highly polluted areas (hot spots) are commonly remediated by excavation or other in-situ and ex-situ techniques. The surrounding areas can remain, however, diffusely contaminated. The excavation, removal and waste treatment of such low contaminated areas would be disproportionately expensive. Biochar, a product from biomass pyrolysis, can be used to treat such low level contaminations by immobilizing contaminants and participating to the ecological stabilization of such sites. In addition, compost has previously been proposed to increase biodegradation of organic contaminants by enhancing soil bioactivity. Hence, the use of biochar for the immobilization of organic and inorganic contaminants may be complemented with the use of compost to enhance microbial degradation of organic contaminants. With the aim to assess this combined remediation approach, a large set of experiments was carried out to investigate (i) the parameters driving the sorption of a wide range of organic contaminants (neutral and acids) to biochars and (ii) how the addition of compost and biochar affect degradation in soils. Results showed that the sorption of organic contaminants to biochars can be predicted using the atomic O/C ratio and the specific surface area of biochars, if those parameters are combined with lipophilicity descriptors for the organic contaminants (K_{HW} and D_{OW} for neutral [1] and acidic contaminants [2], respectively). Two soils were spiked with six different organic contaminants and two heavy metals. The soils were amended with compost and biochar; both amendments changed microbial composition of fungi as well as archaea communities. The degradation of organic contaminants increased upon compost addition but significantly slowed down after additional biochar addition. Overall, investigations on sorption of organic contaminants and the interrelation of sorption with degradation indicate that (i) biochar can be used to immobilize a large range of organic contaminants, and (ii) compost may additionally be used to enhance degradation of organic contaminants that are not immobilized by the biochar. References: [1] M. Kah, H. Sun, G. Sigmund, T. Hüffer, and T. Hofmann, Bioresour. Technol., vol. 214, pp. 225–233, 2016. [2] G. Sigmund, H. Sun, T. Hofmann, and M. Kah, Env. Sci. Technol., vol. 50, pp. 3641–3648, 2016.

TU167

Degradation of estrogen hormones by fungi in wastewater

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Environmental impacts caused by organic pollutants are mostly of anthropogenic origin and of great economic interest due to their persistence and serious effects on human and animal health. Endocrine disrupting compounds (EDCs) are a group of environmental pollutants known for their negative effects due to their high toxicity and persistence and are considered important in the study of organisms that have the capacity to reduce these substances in the environment. According to their activities, the most studied compounds are estrone, 17β-estradiol, 17α-ethinylestradiol besides industrial compounds often found in effluents from sewage treatment plants. Because of the ability to degrade lignin through a non-specific enzyme system, basidiomycetes fungi have great potential for use in bioremediation processes of persistent organic pollutants. *In vivo* and *in vitro* experiments with purified enzymes proved that ligninolytic enzymes are capable of degrading extensively Endocrine Disruptors. Studies shows that the application of isolated enzymes or ligninolytic fungi can biodegrade these compounds through the production of enzymes responsible for the degradation of aromatic compounds. This project aims to investigate the ability of *Trametes versicolor* fungus cultures to degrade typical representatives of EDCs: 17α-ethinylestradiol and estriol. The degradation potential of the *T. versicolor* species was evaluated from the incubation of the fungal mycelium in Enriched Medium, where the standards were injected in flasks with different periods of mycelial growth (7 and 14 days). Aliquots of the medium were removed after 24 and 48 hours of addition of the standards, and enzymatic analyzes were performed via a spectrophotometer and degradation analysis using High Performance Liquid Chromatography (HPLC). In general, the samples had an excellent degradation potential, reaching 100% degradation in the 14 day aliquots, however interference with the Estriol standard was observed in the enzymatic production of the fungus. When comparing the degradations of the two standards, it was possible to identify a better result with the 17α-ethinyl estradiol standard, where samples with 7 days of incubation were analyzed after 48 hours and presented degradation of 98% of the injected standard. The best degradation performance of this hormone may be related to the amount of enzymes produced.

TU168

Set of screening assays as tool for evaluation of bacterial bioremediation potential for polycyclic aromatic hydrocarbons (PAHs)

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The toxic effects of petroleum hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs) are cumulative, while some effects are carcinogenic, mutagenic or teratogenic, so therefore can endanger the health of future generations, and these compounds are considered as potential health and environment risks. When in the environment, PAH compounds are subject to numerous physical and chemical changes, but the most intensive transformations occur as a consequence of microbial action. These processes are frequently used in bioremediation procedures which use microorganisms directly from contaminated sites. To show that a bioremediation procedure is potentially useful, it is important to demonstrate the ability of selected microorganisms to enhance the rate of hydrocarbon degradation in controlled conditions. We compared bioremediation potential for PAHs of 8 pure bacterial isolates obtained from an environment contaminated with petroleum and its derivates, using 3 simple screening methods. Eight hydrocarbon-degradation profiles of pure cultures (*Planomicrobium sp*. RNP01, *Micrococcus sp.* RNP02, *Staphylococcus sp.*RNP03, *Micrococcus sp.*RNP04, *Rhodococcus sp.* RNP05, *Staphylococcus sp.*RNP06, *Planococcus sp.* RNP07 and *Micrococcus sp.* RNP08) were exhaustively investigated using an hydrocarbon growth assay, 2,6 DCPIP assay and dehydrogenase activity assay. *Rhodococcus* sp. strain RNP05 showed the best potential for utilizing the examined hydrocarbon substrates and achieved the maximum value for dibenzotiophene (OD_{600} 0.100), which was confirmed by gas-chromatograph analyses. The use of this combination of all three methods to assess bioremediation potential appears to be suitable for practical work and assures that the best microbial candidates for bioremediation are chosen.

TU169

Screening of mineral media components for enhancing carbazole degradation by Pseudomonas aeruginosa (RS1) using Plackett-Burman design

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Carbazole is a toxic and mutagenic nitrogen (N) heterocyclic polyaromatic hydrocarbon having a dibenzopyrrole structure. It is naturally present in shale oil, coal tars and petroleum products and in wastewater discharged from various industries. Biological treatment of such wastewater may be feasible when reactors are seeded with microbes capable of degrading carbazole. Such microbes can flourish in a bioreactor when their nutrient requirements are satisfied. In this work, a *Pseudomonas aeruginosa* RS1 strain isolated from oily sludge was evaluated for its carbazole degrading ability and the mineral media components were screened for enhancing carbazole degradation using the Plackett-Burman design. Although the Plackett Burman design has been used for designing media for other applications, it has not been previously used maximizing the degradation of any N-heterocyclic compound, such as, carbazole. A total of 16 mineral media components, i.e., KH_2PO_4, K_2HPO_4, $Na_2HPO_4.2H_2O$, NH_4Cl, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $FeCl_3$, $CoCl_2.6H_2O$, H_3BO_3, $Na_2MoO_4.2H_2O$, $CuCl_2.2H_2O$, $ZnCl_2$, $NiCl_2.6H_2O$, $FeCl_2.4H_2O$, $MnCl_2.4H_2O$ and Na_2SO_4 were screened using a high and low level for each component chosen based on a range of media composition reported by various researchers. Carbazole grown inoculum was spiked in culture flasks containing 100 mg/l carbazole and extent of degradation was quantified after 8 days. Six media components namely, K_2HPO_4, $Na_2MoO_4.2H_2O$, $MnCl_2.4H_2O$, Na_2SO_4, $NiCl_2.6H_2O$ and $CaCl_2.2H_2O$ were found to be significant based on the half-normal plot, pareto chart of effects and the percent contribution value of each media component generated using the Design Expert software. Carbazole carry-over with inoculum was observed based on controls, hence initial carbazole concentration was corrected before determining %carbazole degradation. Three media components, i.e., $MnCl_2.4H_2O$, $NiCl_2.6H_2O$ and $CaCl_2.2H_2O$ had the most significant influence on carbazole degradation. Carbazole degradation increased from 37.633 ± 3.518 % in unoptimized media to 58.443 ± 9.39 % in the optimized media which contained $MnCl_2.4H_2O$ at the high level and both $NiCl_2.6H_2O$ and $CaCl_2.2H_2O$ at their low levels and all others at the centre point level. The verification tests performed confirmed the Plackett-Burman test results. Thus, supplementing wastewater with appropriate concentration of nutrients can have a significant influence on carbazole degradation.

Challenges in Assessment and Management of Cosmetics and Personal Care Products (P)

TU170

Endocrine disrupting potentials of six benzophenones using combined in vitro assays

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Benzophenones are frequently used as UV protection agents in personal care products, and also added to plastic packages to prevent photo-degradation of the plastic polymers. Although endocrine disrupting effects of some benzophenones have been documented, there are still significant information gaps across different types of benzophenones. A series of *in vitro* assays employing a human breast (MVLN), a human adrenal (H295R), and a rat pituitary (GH3) cells, were conducted on six benzophenones, i.e., benzophenone, benzophenone-1, benzophenone-2, benzophenone-3, benzophenone-4, and benzophenone-8, and their endocrine disrupting potentials were compared. The test doses for each benzophenone applied were determined based on preliminary cytotoxicity assays for each cell line. In MVLN cell line, benzophenone-1 and -2 showed clear estrogen receptor agonistic transactivation. Benzophenone-1 and -2 also significantly increased relative E2 concentration, i.e., E2/T ratio, in H295R cell line. Both benzophenone-1 and -2 were determined to be most estrogenic compounds among the test benzophenones. In GH3 cell line, *tshβ* gene was significantly downregulated by benzopheonone-2 and -8, suggesting their thyroid disrupting potential through hypothalamus-pituitary-thyroid axis. Our *in vitro* observations show that benzophenone-1, -2, and -8 are most potent endocrine disrupting chemicals among the test compounds. Studies for *in vivo* responses and their environmental health implications of these potent benzophenones are warranted.

TU171

Reasons for Rapid Alert of Personal Care Products

U. Klaschka, University of Applied Sciences

The Rapid Alert System (RAPEX) is a tool to inform EU-member countries and the public about consumer products which do not fulfill the legal requirements and which pose a serious risk to the safety and health of consumers. The reasons why personal care products were identified in RAPEX between 2006 and 2015 are compiled and analyzed. 99% of the 724 personal care products which were notified in that time span pose a serious chemical risk as they contain substances which are prohibited or restricted according to the European Cosmetics Regulation. A large number of these substances is not only highly toxic for human health, but also dangerous for the aquatic environment, such as metals (e.g. lead, mercury or cadmium), preservatives (e.g. methyldibromo glutaronitril or formaldehyde), phthalates (e.g. dibutylphthalate), peroxides (e.g. sodium perborate), aromatic hydrocarbons (e.g. aminophenol), phytotoxins (e.g. *Aconitum napellus*, or *Strychnos*), glucocorticoids, phenylenediamines, acrylates, N-nitrosodiethanolamine, ammonia, thioglycolic acid, methanol, or diethylene glycol. 85 of the notified products are contaminated with potential pathogenic microorganisms, e.g. *Pseudomonas aeruginosa* or *Burkholderia cepacia*. The data show the importance of marketing surveillance.

TU172

IFRA Environemntal Standards And RIFM Program Advances update for 2017

A. Lapczynski, RIFM / Environmental Science; D.T. Salvito, Research Institute for Fragrance Materials (RIFM) / Dept of Environmental Science; C. Gonzalez, IFRA; M. Vey, International Fragrance Associations

To assure safety of fragrance ingredients in consumer products, International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environment Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the IFRA Environmental Standards, RIFM reports the most recent results of these additional tests (for both risk and hazard assessments) at both the annual SETAC NA and Europe meetings. These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented.

TU173

Multigenerational effects of organic UV-filters on the aquatic midge Chironomus riparius

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Organic UV-filters are compounds used in a wide variety of products, such as sunscreens, to protect against ultraviolet radiation. UV-filters are largely detected in aquatic environment reaching concentrations of up to 5.79 μg/L and 2.4 mg/Kg (dry weight) in rivers' waters and sediments respectively. Given the continuous release of UV-filters in the environment and their physic-chemical properties such as high lipophilicity and slow degradation in natural ecosystems, studies evaluating the long-term effects of UV-filters in benthic aquatic organisms are essential. Moreover, it has been shown that UV-filters induce endocrine disruptive effects in both vertebrate and invertebrate species. As such standard partial life-cycle tests can underestimate the toxicity of UV-filters for natural populations. In this study we present the results of a multigenerational experiment where *C.riparius* were exposed to a gradient of Benzophenone-3 a widely used UV-filter. A gradient of environmentally relevant concentrations of BP3 spiked in sediments was used and development time, emergence rate and weight of emerged adult midges were assessed in parental generation and in F1 generation. Also, the fecundity and fertility were evaluated in the parental generation. Results are discussed comparing the impairment of development, and reproductive endpoints of both the parental and F1 *C. riparius* generations under BP3 exposure. This study highlights the validity of these multigenerational experiments for the ecological risk assessments of personal care products and the challenges of using *C. riparius* as a model organism for such an approach.

Environmental Fate, Effects, and Risk Assessment of Veterinary Medicines (P)

TU174

Adsorption of Veterinary Medicines to Soil: Regulatory Limitations of the Koc

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Department of Veterinary Medicines
The requirements for the marketing authorisation for Veterinary Medicinal Products (VMP) are primarily laid down in Directive 2001/82/EC. This Regulatory work frame requires, among other conditions, environmental risk assessment (ERA) analysis to determine the level of risks for the environment. The data package required by harmonized guidelines to carry out an ERA for VMP includes the study of the sorption to soils of the active substance (AS). The resulting Koc (Sorption coefficient -Kd- normalized to the amount of organic carbon) will be used in the ERA to determine the exposure of the substance to surface water, sediment and groundwater. Understanding the processes of sorption when predicting fate and exposure to different compartments is essential to obtain reliable predictions of environmental concentrations (PEC). The sorption to soil of chemicals is a complex process that entails many factors involving sorbant (soil) and sorbate (AS). Among the factors related to the sorbate the structure and functional group(s) play a major role in the sorption behaviour. Within the factors dependant on the sorbant, the most important are: pH, ion strength, amount of organic matter and amount of clay. From the regulatory point of view the Koc is used in a stepwise approach to calculate PECs firstly using simple equations. If further refinement is required FOCUS PEARL or SWASH models are used. All FOCUS models allow the use of Koc or Kom (*organic matter*). In some models the Kd can be implemented but Koc is recommended in the guidelines relevant for VMP. Nevertheless, sorption depends on the properties of the AS and on other factors linked to soil -organic carbon content being only one of them. In the case of ionisable substances the sorption is affected by clay, multivalent metals and organic matter. In these cases, assuming that the sorption is only affected by organic matter would lead to an unrealistic prediction of exposure in ground/surface waters and sediment. Besides, the amount of organic matter decreases with depth, so a substance whose sorption is influenced by clay or metals might leach to lower extent. To summarize, normalization by organic carbon might not be the only important normalization parameter for Kd, especially when dependence on clay content is observed. A harmonized regulatory guidance to apply this concept in the ERA would greatly improve the reliability of the predictions and be beneficial for both stakeholders and regulatory authorities.

TU175
Developing Geographical Scenarios to Assess Environmental Risk of Veterinary Medicines in Europe
C. McMillan, G. Hughes, Cambridge Environmental Assessments
The environmental risk assessment framework for assessing veterinary medicines used to treat livestock and registered in Europe, follows a tiered approach for surface and groundwater. The initial exposure assessment is a simplistic approach with refinement using the FOCUS suite of models (FOrum for Co-ordination of pesticide fate models and their USe) for surface and groundwater often required. The refinement option comprises a conservative application scenario for assessing surface water exposure (6 drainage and 3 runoff scenarios) and groundwater exposure (1 scenario) and is intended to provide a realistic worst case assessment of environmental exposure from veterinary medicine usage in livestock. However, in applying a modelling framework designed to be broadly representative of pesticide usage in arable agriculture at an EU scale, a degree of realism is lost in the veterinary medicine risk assessment process. One way of addressing the deficiencies in the current system could be to move to ‘zonal’ scenarios that more closely represent geographical areas in Europe where livestock husbandry, farming practices and climate are similar. This study considers a zonal approach drawing parallels with that used for plant protection products. The relevance of the FOCUS scenarios in such a ‘zonal’ approach will be considered, alongside other factors like animal husbandry, and its utility in assessing veterinary medicine risk to surface and groundwater.

TU176
Development and validation of a draft test protocol for studies on transformation of veterinary pharmaceuticals and biocides in manure
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The spread of veterinary medicinal products (VMP) and biocides onto agricultural land represents a very important path of entry into the environment for these product groups. For this reason, current guidance (e.g. „Guideline on determining the fate of veterinary medicinal products in manure“ (EMA/CVMP/ERA/430327/2009) stipulates experimental studies on transformation of VMPs and biocides in manure. The documents only contain basic regulatory requirements, an experimental test guideline is still missing, both on EU and OECD level. Therefore, to allow for a consistent assessment of studies within the authorization process, a harmonized internationally accepted and validated test method is needed. Within this context, a test protocol for an experimental method to study the transformation of veterinary pharmaceuticals and biocides in cattle and pig manure was developed. The test design of established simulation type studies (e.g. soil and water-sediment systems) was adapted to the specific requirements of the matrix manure. In the first project, the experimental method was adapted, examined and revised by an intra-laboratory comparison using various test compounds and types of manure. In a second step, an international inter-laboratory comparison (pre-validation ring test) was performed to collect experiences of the participants and to revise the test protocol as required. In the follow-up project, an international validation ring test has been performed, testing a veterinary medicinal product (florfenicol) in pig manure and a biocide (imidacloprid) in cattle manure. In two international workshops, the ring test results from all the participants were discussed with international experts. In this context, technical details of the draft test protocol were reviewed to increase clarity and unambiguity. Based on the outcome of this ring test, it was concluded that the draft test protocol proved to be robust and applicable although using partly different experimental set-ups in different laboratories. Therefore, the experimental method is considered to be well-suited to examine the anaerobic transformation of veterinary pharmaceuticals and biocides in liquid manure. In the next step, the final version of the draft test protocol is intended to be submitted to the OECD Test Guidelines Programme. The poster presents the results of the international validation ring test and the main conclusions drawn from both research projects (for reports see www.umweltbundesamt.de).

TU177
Proposal for a 'standard' field study for the evaluation of the effects of parasiticides on dung and soil organisms
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For more than 20 years the ecological effects of Veterinary Medicinal Products (VMPs) on dung-associated organisms have been studied in the field, most of the time with the parasiticide ivermectin. Regularly, effects on the structure (i.e. biodiversity) and functions (e.g. dung degradation) of the dung organism community have been found at realistic exposure scenarios. In addition, soil organisms could be affected when feeding on dung containing VMPs. According to European legislation, veterinary medical products (VMPs) have to be authorized before they can be put on the market. During this process, their effects on dung and soil organisms have to be assessed based on the results of single-species toxicity tests, which are performed according to OECD standards. In case of adverse effects on, for example, dung flies, dung beetles, earthworms or springtails, higher-tier tests (i.e. mainly field studies) could be required. For these reasons, an international research project was undertaken to develop and validate a proposed field test method under varying conditions of climate, soil, and endemic coprophilous fauna at Lethbridge (Canada), Montpellier (France), Zurich (Switzerland), and Wageningen (the Netherlands). The specific objectives were to determine if faecal residues of an anthelmintic with known insecticidal activity (ivermectin) showed similar effects across sites on: (1) insects breeding in dung of treated animals, (2) coprophilous organisms in the soil beneath the dung, and (3) rates of dung degradation. The focus of this poster presentation is on the lessons learned from a methodological point of view. In short, the test method used in this project does fulfill the requirements of a higher tier field test as, for example, used in the context of pesticide registration. Detailed recommendations can be given for the information required for the selection and description of the study site, the design of such studies (e.g. regarding the number of replicates), the application of the test substance (including the minimum requirements of analytical verification of exposure), the most important structural and functional endpoints as well as the methods to measure them in the field. Experiences from related field studies (e.g. as part of monitoring programmes) have also been considered when summarising these recommendations. A guidance for the performance of such tests, also based on the results of this research project, has recently been published for public consultation by the European Medicine Agency.

TU178
Higher-tier, modelling-based refinements for environmental impact assessments of veterinary medicinal products used as bath treatments in fish farms
J. Carnall, F. Ericher, G. Hughes, Cambridge Environmental Assessments
Diseases of fish populations, such as infestations of sea-lice, are often treated in commercial fish farms using solutions of veterinary medicinal products (VMPs) applied topically, *via* bath treatment. Typically, for open system farms, a tarpaulin is placed temporarily around a cage of fish; this encloses the treatment solution, which is subsequently discharged into the surrounding waters at the end of the treatment period. \n For VMPs applied in open system fish farms *via* bath treatment, a Phase II environmental impact assessment is required as part of the EU

registration process. This assessment must include the calculation of Predicted Environmental Concentrations in surface water ($PEC_{surfacewater}$), which are subsequently compared with Predicted No Effect Concentrations (PNECs) derived from environmental effects studies. $PEC_{surfacewater}$ are typically calculated using models developed by the Scottish Environment Protection Agency (SEPA) to assess exposure in the water column. The SEPA models calculate dilution, dispersion and degradation of a chemical patch in the hours and days following its discharge. The calculations performed by the SEPA models are necessarily simple in order to limit the need for site-specific survey work in determining the model inputs. The simplicity of these calculations, however, can for some compounds lead to a divergence between the model predictions and expected exposure patterns in the environment. In this paper, we discuss higher-tier refinements for the calculation of $PEC_{surfacewater}$ using the SEPA models, following the use of VMPs in fish farms.

TU179
On the veterinary pharmaceuticals and crop protection substances: data availability, hazard identification and knowledge gaps
S. Kools, T.L. ter Laak, KWR Watercycle Research Institute
Risk assessment on compounds starts with assessing data on occurrence, fate and hazards. In the Netherlands (and other countries), authorities rely heavily on the availability of good data on compounds in waters, especially in the framework of ecological and human health. In the last of years, much attention has directed towards the subject of emerging compounds. Here, many pathways of entry into the environment have been identified. Industrial chemicals are reviewed, some agricultural sources have been identified and household usage of chemicals has been recognized. In particular, the usage of compounds for agricultural and veterinary use have been getting some attention. Yet, questions remain on the extent of use and insight in the (presumed) widespread use is not known in detail. In this presentation, data was collected from different sources on the (nation-wide) occurrence of veterinary pharmaceuticals and pesticides. Also, we aim to include data on the potential for the spread into the environment and hazards using existing data. For water managers and drinking water companies in particular, the issue on the unknown emissions may be relevant in the context of the attention of emerging compounds (and their transformation products). Also, we compare the available occurrence data to the identified hazards to gain more insight in the extent of the issue. From this project, we identified knowledge gaps and future research needs.

TU180
Risk based Environmental Assessment of groundwater ecosystems related to use of veterinary medicinal products
A. Finizio, University Milano - Bicocca / Department of Environmental and Lanscape Sciences and Earth Sciences; B. Kolar, National Laboratory of Health, Environment and Food
Before any new veterinary pharmaceutical product (VMP) can obtain marketing authorization, a thorough analysis has to be carried out by national and/or European Union (EU) authorities regarding its efficacy and risk to human health and the environment. For the environmental risk assessment (ERA) of VMPs in groundwater (GW), the current EU guidance documents (VICH guidelines) suggest an approach based on the comparison between the calculated concentration in GW (PEC_{gw}: Predicted Environmental Concentration in groundwater) and an arbitrarily set threshold concentration of 0.1 μg/L. The latter is the upper limit of the concentration for pesticides in drinking water in the EU. If the calculated PEC_{gw} does not exceed the threshold of 0.1 μg/L, then the risk is considered acceptable. On this basis, it is not clear whether the GW is recognized as an aquatic ecosystem or just as a source of drinking water. In fact, it is assumed that the concentration of 0.1 μg/L is by default safe for both humans and exposed GW organisms. However, there is increasing evidence that the biodiversity in GW ecosystems can be much higher than previously thought and in some cases, could even exceed the biodiversity of surface ecosystems. This largely unrecognized biodiversity is worthy of environmental protection through the adoption of a more scientifically sound risk analysis, which should be based on the consideration of ecological criteria that are thus far not taken into account for GW systems. Based on the evidence of the peculiar characteristics of GW ecosystems and their vulnerability, it is proposed that assessments of GW ecosystems will be risk based and become a compulsory part of the overall risk assessment of VMPs. It is suggested the use of a risk quotient approach based on the PEC/PNEC ratio in which the PNEC (Predicted No Effect Concentration) is calculated including an additional safety factor of 10 to the calculated PNEC for surface water systems (PNECgroundwater = PNECsurfacewater/10). In this study, we highlight and discuss the scientific basis underlying not only the environmental risk assessment of GW ecosystems exposed to VMPs but as well feed additives, plant protection products, biocides and other chemical substances under REACH legislative framework.

TU181
SETAC Interest Group on Dung Organism Toxicity Testing
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Interpreting Biological Effects of Metals and Their Mixtures in the Aquatic and Terrestrial Environment (P)

TU182
Multiple Stable Isotope Analysis for the Assessment of Pollution Sources in a Mangrove Food Web
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Metal contamination is a long-established problem in mangrove ecosystems. However, only few studies have focused on the source of contamination at different levels within the associated food web. Thus, our main goal was the evaluation of the structure of a mangrove food web looking for probable sources of pollution that can affects two estuarine ecosystems in the state of Espírito Santo, Brazil (Santa Cruz and Vitória Bay). Four stable isotopes (carbon, nitrogen, strontium and lead) were analysed in the sediment, trees, plankton, shrimps, crabs, oysters and fish. In addition, strontium and lead isotopic ratios were also analysed in surface water and particulate matter (PM) of the Tubarão Harbour Complex, the main industrial activity in this area. Carbon isotopes identified the primary producers, indicating that plankton and trees are basal starting species within the studied food web. As expected, nitrogen isotopic signature were increased as the trophic levels upturn, allowing the assessment of crab, shrimp and oyster as intermediate levels within the food web, while fish was confirmed at the top level. Nitrogen isotopes also indicate differences between both estuaries. Namely, trees from Santa Cruz use ammonium as the main nitrogen source, while trees from Vitoria Bay faced anthropic effects, with nitrogen source resulting from fertilizers, which causes an increase in $\delta^{15}N$ for higher trophic levels of this food web. Strontium isotopic ratios show the greater influence of the marine water rather than continental sediment throughout the entire food web, including mangrove trees. Results from the different lead isotope ratios suggest that all sources of contamination have been strongly influenced for metallurgical activities. Moreover, lead isotopes ratios found in sediment had values higher than those previously reported. Additionally, the lead isotopic signature corresponding to particulate matter was also found in surface water, trees, plankton, crab, shrimp, oyster and fish from both studies estuaries. This last result point out the particulate matter as the main responsible for metal contamination in both mangrove ecosystem. Since all food web showed a strong nutritional influence of surface water and that the most probable source of contamination in this ecosystem is the particulate matter that chronically leave metals available in surface water, the effect of this source can be amplified by the dynamics of this ecosystem.

TU183
Biogeographic response of Emiliania huxleyi to Cu: the Humboldt Current System case
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Phytoplankton and metals have co-evolved throughout the history of the global oceans, the former adapting to available trace nutrients and metals modulating the macro-nutrient fluxes by co-regulating phytoplankton growth. An essential but potentially deleterious metal to phytoplankton is Cu, both at deficient and excess levels. To limit these impacts, phytoplankton is able to sequester Cu inside the cell by organic chelation, release organic complexing ligands and increase Cu efflux processes. In this study, we investigated whether the sensitivity to Cu-inhibition of growth of *Emiliania huxleyi,* the most widespread marine calcifier, varied by origin, comparing a total of 17 strains isolated from coastal and open ocean waters of the Southeast Pacific, the Mediterranean Sea, and the Tasman Sea. We analyzed specifically whether sensitivity related to predicted Cu inputs, contrasting coastal vs open ocean strains responses, or latitudinal gradients. Finally, we analyzed whether production of organic Cu ligands related to sensitivity to growth inhibition by Cu. Most of the HCS strains showed significant decreases ($p< 0.05$) at concentrations above 200 nM Cu, although for some of them, this significant decrease was only observed at 600 nM Cu. Mediterranean strains showed significant decreases ($p< 0.05$) starting at 300 nM Cu but, interestingly, populations exposed to 50 and 100 nM Cu experienced significant increases ($p< 0.05$) in their growth. No significant differences ($p>0.05$) were observed between different morphologies, i.e., regarding their calcification state. However, a slight trend was observed as strains tended to be more calcified, i.e., from the less calcified morphology B/C to the heavily calcified morphology HC. Coastal strains were more tolerant than offshore strains to Cu addition and produced more CuL with respect to their EC50, correlating to the recurrent inputs of atmospheric Cu (not observed for the offshore strains). The higher latitude strains were more sensitive to Cu than those closer to the Equator, which were those producing higher amounts of CuL.

TU184
TOXICITY AND BIOCONCENTRATION OF HEAVY METALS IN SEA

STARS ASTERIAS RUBENS: MECHANISTIC APPROACH AND MODELING

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The growth of anthropogenic substances getting into the environment makes it difficult to evaluate the level of pollution, its changing over time, as well as optimization of the environmental and control activities. This issue is especially relevant to marine ecosystems due to the fact that the potential toxic effects of pollutants in marine environment and marine organisms are significantly different from those effects observed in fresh water, and are not yet studied enough. Thus, it is necessary to organize the monitoring of marine environments, the search and investigation of target values, including bio-indicators and representative bioassays. Promising research objects refer to the cells of the coelomic epithelium and coelomic fluid of Asterias rubens Linnaeus starfish, 1758 as primary or immortalized cell lines. High ecological plasticity of Asterias rubens starfish favors its wide population and resistance to anthropogenic influence and explains the potential of using those species as model organisms across large areas and in conditions of high anthropogenic load levels. During the toxicological experiments with heavy metals changes at the level of cells of the coelomic fluid of starfish *Asterias rubens* were described, at the end of the experiment the content of heavy metals in the bodies of the starfish was determined. Copper is the most toxic metal with its high concentration provoking death of Asterias rubens starfish. The value of coelomocytes of various subpopulations is defined by the way of adaptation of starfish to a particular metal (metal accumulation in the body or its elimination). The ability to bioconcentrate decreases in the order Pb>Cu>Fe>Mn>Cd>Co. The high adaptability of starfish at the level of coelomic fluid cells is revealed and registered through absence of differences between the number of cells in experimental aquaria with high metal concentration and the control group. When speaking about the effect on the distribution of cell subpopulations, metals can be divided into two groups – those accumulated in the body showing the increase in the proportion of granulocytes, and those metals that are not accumulated showing the increase in the proportion of agranulocytes. Bioconcentration is more likely determined by the ability of metals to build insoluble complexes (hydrolyses constant) in sea-water.

TU185

Assessments of bioavailability and mixture toxicity of zinc, copper, and nickel in Japanese surface waters using modeling approaches

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We assessed the mixture effect of zinc, copper, and nickel by combining mixture models (concentration addition and independent action, CA and IA), species sensitivity distribution (SSD), and chronic biotic ligand models (BLM). We applied these models to Japanese water quality data as a case study. First, water quality data in 182 Japanese surface waters were obtained from the Database of Water Quality of Aqueduct managed by Japan Water Works Association. Water hardness, pH, and organic carbon concentration ranged from 5 to 172 mg/L (as $CaCO_3$), 6.0 to 9.3, and 0.05 to 4.9 mg/L, respectively. The chronic toxicity database we used includes NOEC or EC10 values for 22 species (128 test results) for zinc, 28 species (135 test results) for copper, and 31 species (214 test results) for nickel. Then, these chronic toxicity data were normalized using chronic BLMs and the water quality data in each site. After that, SSD analyses were conducted for each metal and each water body. The site-specific 5 percentile of SSD (HC5) for zinc, copper, and nickel ranged from 5.6 to 40.7, from 0.2 to 22.0, and from 0.6 to 16.6 μg/L, respectively. Organic carbon concentration was the most important factor affecting the metal toxicity. Finally, CA and IA models were applied to site-specific SSDs to calculate mixture toxicity as a multi-substance potentially affected fraction (msPAF). The median values (across the whole Japanese monitoring dataset) for msPAF by the CA and IA models were 0.138 and 0.083, respectively. The values of msPAF were mostly determined by copper toxicity. The CA model resulted in a more conservative output (i.e. a higher msPAF) than the IA model.

TU186

The investigation of the ecological vulnerability of aquatic organisms for cadmium

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Although the traditional risk assessment approaches have played a key role, the extrapolation from individual response to ecosystem response and the exclusion of interaction among the organisms are still criticized. Assessing the vulnerability of ecosystem is the approach to predict and judge the state of overall ecosystem by summing the innate traits of organisms and the composition surrounded. In addition, the accumulation potential is considered by assigning different weight depending on the trophic level. As a result, the more extensive observation of organism is allowed for its population within the ecosystem. In this study, the ecological vulnerability of aquatic organisms was observed for the exposure potential of cadmium in Korean freshwater. To display the vulnerability, organisms' innate traits were investigated, quantified scores by using multi-criteria analysis, and assigned the weight factors through multi-variate ordination analysis. The derived score and weight factor on each trait were integrated to the quotient representing the numerical vulnerability of species. Consequently, the vulnerable species for cadmium were found to the species which generated the greater numerical vulnerability. The vulnerable species were compared with the sensitive species, and the impact of traits other than toxic response to the organisms was confirmed. This subject is supported by Korea Ministry of Environment(MOE) as "The Chemical Accident Prevention Technology Development Project".

TU187

Molecular Toxicity of Metal Mixture and Defense Mechanisms in Zebrafish (Danio rerio).

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Metals play a pivotal role in various biochemical and physiological processes. They are widely found in nature, particularly in mineral deposits, soils and sediments. Virtually, all metals, including the essential metal micronutrients, are toxic to aquatic organisms if exposure levels are sufficiently high. The toxicity of the most important metals such as Cu, Zn, Ni, Pb, Cd, etc have been extensively documented for various species and the results are used to derive environmental standards. Most of the studies on metal toxicity consider the effects of single metals with little attention to mixture scenarios. In the natural environment the organisms are exposed to different metal compounds simultaneously, thus making it necessary to study combined metal exposures. In this perspective, we studied the toxicity of mixed metals and the underlying molecular mechanisms to provide new insights in generic versus metal specific stress responses. This study aimed to understand how exposures in a single and multiple metal pollution contexts differentially affect the zebrafish. In this experiment, fish were exposed to 50µg/L of Cu, 25µg/L of Cd and a 50+25µg/L Cu+Cd mixed dose for a period of 7 days. Fish gills, gut and liver were collected for metal analysis by ICPMS and gene expression of selected genes by qPCR. 16 genes were selected related to energy metabolism, oxidative stress, apoptosis, membrane transport and DNA damage and repair. We observed a strong response of genes in both single and combined exposures. Out of the total of 16 genes, 14 genes responded to the combined exposure in gills, 11 in gut and 14 in liver. A notable up regulation of Catalase, MT2, HSP70, SOD1, SOD3 was observed in different organs. Also an interesting response of DNA damage and repair genes Gadd45 and Rad51 was observed in mixed exposure in gill and liver which suggests a possible impact on DNA. Overall there was clear evidence that combined metal exposure resulted in synergistic response profiles at the gene expression level.

TU188

Binary metals mixtures effects on spring barley Hordeum vulgare nutritional status

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Heavy metal pollution is considered as one of the most serious problems worldwide and has significant environmental and human health impact. Copper and cadmium contamination is widespread due to their intensive industrial and agricultural use. Maximum permissible levels of heavy metals in the environment are regulated by a number of existing guidelines, however these guidelines regulate single elements threshold concentrations. Whereas metals are always found in the environment in the mixtures rather than as single elements. Within the last several decades scientific and regulatory concern over the ecological effects and risks of chemical mixtures has increased. The present study aimed at investigating the effects of binary mixtures of copper (Cu) and cadmium (Cd) on the nutritional status of spring barley (*Hordeum, vulgare* L.). The seedlings of barley were treated with single Cd and Cu (ranging from 0.1 to 100 mg L^{-1}) and with binary mixtures of these metals. Binary mixtures toxicity was evaluated using toxic unit (TU) approach. Single and combined metal treatment led to major effects in the growth of barley, altered the content of photosynthetic pigments and induced lipid peroxidation. The uptake and accumulation of Cu and Cd was considerable influenced by the interaction among these metals. Analysis of micronutrient content in the plants revealed that binary mixtures of Cu and Cd impaired the barley's nutritional status. In this poster we discuss single and joint Cu and Cd interaction with key micronutrients content.

TU189

Cu and Cd affect distinctly the biochemistry of two ubiquitous tropical green algae

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Brazilian wetlands cover ~20% of Brazil's territory and are highly vulnerable due to the intense overexploitation of its natural resources. In these ecosystems, phytoplankton is responsible for primary production, affecting the chemistry and cycling of many bioaccumulative chemicals, including Cu and Cd, two of the most widespread trace metals derived from human activities. Although the sources, fate and effects of these metals have extensively been studied in temperate regions, their influence and toxicity to tropical organisms remains vastly unexplored. The present research examined the impacts of environmentally relevant concentrations of Cu and Cd on the biochemistry of two tropical freshwater Chlorophyceae, *Chlorolobion braunii* and *Scenedesmus quadricauda*, by analyzing the concentrations of carbohydrates, proteins, chlorophyll *a* and lipids on cells exposed to a range of metal concentrations. The biochemical composition of both species was altered after the addition of both metals, specially in the case of Cd. Both species showed a constant increase in chlorophyll *a*, proteins, carbohydrates and lipid contents up to a certain metal concentration, beyond which started to decrease. However, when cell size was included into the analysis, it was observed that proteins content remained constant up to a certain threshold, when their production increased; and that Cd induced a significant ($p< 0.05$) increase in carbohydrates production. On the other hand, chlorophyll *a* was reduced within cells, while lipid contents and composition varied depending on the metal tested and the concentrations added.

TU190
Interaction between rare earths and phosphate in the aquatic environment and implications for toxicity testing with algae and aquatic hazard and risk assessment
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Over the past few years, standardised algal growth inhibition tests have been performed with a variety of rare earth compounds in view of REACH registration. Because rare earth elements are known to have an extremely strong affinity for phosphate, resulting in precipitation of highly insoluble rare earth phosphates, phosphate concentrations were carefully monitored during most algal growth inhibiton experiments. When evaluating the results of these experiments, it became clear that in all cases, regardless of the solubility of the substance or the valence of the rare earth, significant growth inhibition was coinciding with phosphate depletion from the test medium. Visual MINTEQ (3.1) equilibrium modelling was performed for some of the experiments and indeed confirmed that all phosphate is depleted from the test medium whenever the rare earth is in excess and vice versa. Good agreement was obtained between predicted and observed dissolved phosphate and rare earth concentrations. Other important observations were that 1) complementation of phosphate levels to nominal levels just before the start of testing completely relieved the effects observed in non-complemented test media, and 2) experiments with phosphate-containing rare earth compounds did not observe significantly different phosphate depletion compared to the control treatment and consequently did not observe any significant adverse effects on algal growth. Existing data in scientific literature were completely in line with these findings: taking into account the phosphate concentration of the test media used, it became clear that adverse effects on algal growth were only observed at rare earth concentrations close to or exceeding nominally added phosphate concentrations in the test media. No experiments have been identified so far in which a direct effect of rare earth elements on algal growth is convincingly demonstrated. The observed effects were clearly due to phosphate deprivation. In this study, the relevance of these phosphate deprivation effects (which have been observed in small-scale test systems) for large-scale aquatic ecosystems was critically evaluated. Further, the implications of abovementioned findings towards hazard and risk assessment as well as environmental classification of rare earth compounds have been thoroughly discussed, taking into account available toxicological information for rare earths in organisms of other trophic levels (e.g., invertebrates and fish).

New developments in ecotoxicology for the risk assessment of single and multiple stressors in insect pollinators: from the laboratory to the real world (P)

TU191
Are there differences in honey stomach extraction of nectar between two different pollinator species?
A. Kling, A. Molitor, E. Verge, Eurofins Agroscience Services Ecotox GmbH; S. Knaebe, EAS Ecotox GmbH / Ecotox Field
The EFSA guidance document on the risk assessment of plant protection products for pollinators was published in 2013. In this document options for a refinement of the risk assessment of exposure are suggested. Therefore, nectar and pollen can be collected either by hand or with the help of pollinators. If pollinators are used for the collection of nectar, usually the content of the honey stomach is used for residue analysis. Mostly honey bees (*Apis mellifera*) are used for this purpose but for special cultures bumble bees (*Bombus terrestris*) can be used as well. For both species a tunnel set-up is used in order to restrict the foragers to a defined area of crop. Thus, different sampling patterns for food are of minor importance. In contrast to the use of honey bees, less labour is needed for the preparation and set-up of bumble bee colonies and consequently the collection of nectar with bumble bees is much easier. Furthermore, the size of the bumble bee honey stomach is usually bigger compared to those of honey bees and therefore less individuals might be necessary to reach the amount of nectar needed for analysis. To have experimental proof of the hypothesis that the extraction of the nectar stomach of the bumble bee is equivalent/similar to the extraction of honey bee stomachs a laboratory experiment was set up. Honey bees and bumble bees were caged in the laboratory and fed with 50% (w/v) aqueous sucrose solution containing a defined concentration of a well known plant protection product. The honey stomachs of both species were extracted and the residue content analysed. The results will be discussed and recommendation given for sampling with bumble bees compared to honey bees.

TU192
Limited solubility of test items in regulatory honey bee (Apis mellifera) testing: potential use of solvents, solubilizers and viscosifiers with aqueous sugar solution
W. Bluhm, Independent Consultant; A. Ehmke, IBACON GmbH; D. Gladbach, BayerCropScience; A. Kling, Eurofins Agroscience Services Ecotox GmbH; S. Wollert, Dr Knoell Consult GmbH; S. Ruhland, BioChem agrar GmbH; D. Sack, BASF SE; S. Schmitzer, IBACON GmbH; M. Theis, Bayer CropScience; N. Hanewald, BASF SE / Ecotoxicology
The European Food Safety Authority (EFSA) has evaluated the current risk assessment of Plant Protection Products (PPPs) on honey bees and published a Draft Guidance Document for the risk assessment on bees (EFSA Journal 2013; 11(7):3295). Gaps in the current testing scheme were highlighted, including chronic exposure of bees. In response to that, an OECD test guideline is currently under development describing a 10-day chronic feeding test in the laboratory to adress a prolonged exposure phase of adult honey bees (*Apis mellifera*). Young worker bees are exposed to the test chemical via 50 % sucrose solution in an *ad libitum* feeding scenario. The test is used to determine LC_{50}/LDD_{50} (lethal concentration 50 %/ lethal dietary dose 50 %) and NOEC/NOEDD (no observed effect concentration/ no observed effect dietary dose) values, which are required for the risk assessment. However, the determination of LC_{50}/LDD_{50} and NOEC/NOEDD values is often limited by the solubility of an active ingredient, rather than its toxicity to the target organism. Therefore, the use of solvents, solubilizers and viscosifiers can be a useful tool to enable testing of test chemical concentrations causing toxic effects on the test organism. So far, limited information on such additives is available with respect to toxicity on the honey bee. In 2015 and 2016 a subgroup of the international ring test for the standardisation of a 10-day chronic feeding test on honey bees in the laboratory (including five laboratories) performed a total of 70 10-day chronic feeding studies. These studies investigated the potential use of solvents (acetone, dimethyl sulfoxide (DMSO), dimethylacetamide (DMAC)), non-ionic surfactants (Tween® 80, Triton™ X-100, Cremophor®), one solubilizer (sodium lignosulfonate (SLS)) and a viscosifier (xanthan gum) in the honey bee chronic oral toxicity test. Furthermore, some combinations of the mentioned additives were tested. Inline with the draft OECD 10-day chronic feeding guideline, preliminary results confirm the use of acetone up to 5 % (v/v). The non-ionic surfactants Triton™ X-100 and Cremophor® as well as the solvent DMAC appear to be unsuitable for honey bee chronic oral toxicity testing due to increased mortality rates already at low concentrations. The results for DMSO, SLS, Tween® 80 and xanthan gum and their combinations indicate a safe use of concentrations not exceeding 1 % (v/v). In several cases the tested additives led to a reduced food uptake.

TU193
Estimating honeybee forager background mortality: a case study in The Netherlands
I. Roessink, Alterra / Environmental Risk Assessment Team; J. Van der Steen, Wageningen Plant Research; D. Belgers, Wageningen Environmental Research / Environmental Risk Assessment Team; H. Baveco, Wageningen Environmental Research; A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; J. Boesten, Wageningen Environmental Research
One of the key assumptions in the EFSA guidance on the risk assessment of plant protection products on bees (2013) concerns the value of honeybee forager background mortality. This background mortality is crucial because its value feeds directly into the trigger value used in the Tier-1 risk assessment. Low forager background mortality results in conservative trigger values, whereas higher forager mortality values result in less conservative triggers. A proper estimate of forager background mortality is therefore key to a realistic and robust risk assessment. Data underlying the current estimate of forager mortality mostly originate from studies performed outside of Europe, with only one European study being available in the city centre of Basel. The value used in Tier 1 (5.3% mortality per day) is the measurement from Basel because this was the lowest value found. Since the city centre of Basel is not representative for European agricultural environments, a new study was performed that was focussed on the estimation of forager mortality in a realistic agricultural setting in the Netherlands. Freshly emerged honeybees (age < 24h) from two hives were tagged every two weeks with micro-transponder RFID chips at the outdoor experimental station 'De Sinderhoeve' . Tagging continued from June to October and every tagged cohort was followed in time. Bees were detected: a) upon tagging, b) when they left the hive and c) when they entered the

hive. First results of data evaluation indicate that already within 1 week some bees left the hive briefly but that foraging commenced usually after two weeks and lasted in individual cases for more than 5 weeks after tagging. Based on the obtained data sets, estimates for the honeybee forager background mortality will be presented.

TU194

Assessment of forager mortality in bumblebees

M. Wang, WSC Scientific GmbH / Dept Efate Modelling; A. Görlich, WSC Scientific GmbH

Lower tier risk assessments of plant protection products for bumble bees conducted according to the current EFSA guidance document (EFSA, 2013) relatively often indicate a high risk to bumblebees. Hence, field or semi-field studies are required for further testing. At present, however, it is uncertain how effects on bumblebees could be best assessed, as there are no established standard procedures for the assessment of hives or of foragers. The assessment of effects on foragers is of particular interest, since recent literature indicates that primarily foragers might be affected by pesticides. We therefore explored to what extent videography may be suitable to monitor pesticide related alterations of foraging activity and forager mortality. We show how these methods could be applied routinely in field or semi-field studies.

TU195

Experiences made with bumble bee testing and the anthranilic diamide insecticide, cyantraniliprole

A. Dinter, DuPont de Nemours (Deutschland) GmbH / Crop Protection; A. Samel, DuPont

Currently there are no internationally adopted test guidelines available for bumble bee testing. The International Commission for Plant-Pollinator Relationships (ICPPR) group on Non-Apis testing is starting to develop test methods for bumble bees. To understand the potential effects of cyantraniliprole (DuPont™ Cyazypyr®), an anthranilic diamide insecticide, several tests with the bumble bee species, *Bombus terrestris*, were conducted following state-of-the-art testing approaches at the time of actual testing. Studies comprised acute contact and oral laboratory exposure studies, greenhouse testing in tomato crop in Spain and semi-field tunnel testing with *Phacelia tanacetifolia* in Germany. In the higher tier studies exposure scenarios via drip irrigation and/or spray application were investigated. The poster will summarize the technical challenges experienced with testing *B. terrestris* under different testing conditions and the effects observed for cyantraniliprole versus the control and the toxic reference treatments.

TU196

Bumble bee acute testing: Why not testing more than one endpoint?

S. Kimmel, Innovative Environmental Services (IES) Ltd. / Ecotoxicology; M. Cornement, Innovative Environmental Services IES Ltd; T. Schmidt, IES Ltd / Ecotoxicology; S. Höger, Innovative Environmental Services (IES) Ltd / Environmental Toxicology

In recent years it became more and more important to add additional pollinator species to the testing scheme of plant protection products which was so far exclusively reserved to honey bees for many years. Therefore, ring test groups were established to develop testing guidelines which are urgently required for the assessment of plant protection products. For the bumble bees acute draft guidelines already exist regarding oral and contact acute testing and taking into consideration the honey bee testing guidelines OECD 213 and 214. The principle of this method is either to expose adult worker bumble bees for 1-4 hours to aqueous sugar solution containing the test chemical (oral testing) or to apply drops of an application solution of the test substance to the thorax (contact testing). After the application of the test substance, the test organisms are provided with 50 % aqueous sugar solution ad libitum and are observed for the following assessments 24 and 48 hours after application. Endpoint is mortality, expressed as LD_{50} and NOED. In addition to the assessment of mortality, the test organisms are checked qualitatively for sub-lethal symptoms of toxicity (affected and moribund condition). In this presentation an additional sub-lethal endpoint is introduced which should not replace the categories of symptoms of toxicity but may give additional information: The consumption of food is a complex behavioral action with several components like recognition of the availability of food, coordinated movement to the food source or coordinated ingestion of the food. All of these components of feeding behavior can be impacted by the test substance and may reduce the amount of ingested food after the application of the test substance. Therefore, the measurement of ingested sugar solution 24 and 48 hours after application is expected to give information on a possible impact of the test item on feeding behavior. Additionally, the assessment of feeding behavior by measuring the consumption of food provides information on possible reasons for mortality (e.g. repellency of the test substance). Acute toxicity tests were performed with *Bombus* spec. using the test substance dimethoate with the special emphasis on the feeding behavior of the test organisms. The results are discussed considering a possible implementation of food consumption as additional information source supporting existing guidelines.

TU197

Bumble Bee Semi-Field Studies - Technical Advances In Data Collection Enable More Rigorous Assessments of Endpoints Including Queen Production Rate and Colony Life

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Semi-field studies of bumble bees are more challenging than honeybees or solitary bees in that (a) brood is awkward to observe without disruption of the nest, and (b) non-destructive determination of numbers of individuals throughout the life cycle of the colony is not straightforward. The authors propose that the following endpoints are valid indicators of impact on bumble bee colonies and describe methods of assessing these endpoints. the rate of bee traffic; the rate of pollen collection; the numbers of queens produced; the number of males produced; the active life of the colony. In order to provide rigorous and valid data, the authors made and field tested multiple units of an integrated apparatus which combines the following functions:- automatic counting of every bee entering and leaving the nest; time-stamping of above data; automatic differentiation between, and recording of, workers and queens; self-adjusting pollen trap to compensate for variable forager size. The apparatus operates throughout the period of the study with no or minimal technician input. The data is securely stored on a conventional SD card. The following additional functions are under development for inclusion in the system:- automatic differentiation between worker and male; automatic detection of every pollen load carried into nest.

TU198

Methods to assess queen reproduction in semi-field trials with bumble bees

L. Franke, J. Fricke, Eurofins Agroscience Services Ecotox GmbH / Ecotoxicology Field; O. Klein, Eurofins Agroscience Services EcoChem GmbH / Ecotox Field; S. Knaebe, EAS Ecotox GmbH / Ecotox Field

Bumble bees (*Bombus terrestris* L; Hymenoptera, Apidae) provide important pollination services and are therefore commercially used, e.g. in greenhouse cultures. Consequently, the impacts of pesticides on bumble bees were already tested in the past. In the light of the newest EFSA guidance document on the risk assessment of plant protection products for pollinators standardized higher tier studies for pollinators are needed. For that reason a ringtest protocol for a bumble bee semi-field study design was developed in the ICPPR Non-Apis working group in 2015 and 2016. The central endpoint in a higher tier bumble bee study is the colony reproduction success (production of young queens). At the end of the annual life cycle of a bumble bee colony workers die and young queens overwinter. Queens that survive establish a new colony in the following year. However, assessing queen reproduction is challenging and the right timing for the termination of the study is essential to obtain a good database for statistical analysis. The study is terminated when colonies start to produce sexuals ("switch-point"), which precedes the natural break-down of the colonies. We tried to answer several open questions concerning queen reproduction as part of the ringtest in 2016. Main questions were: How can the switch-point be determined, which method to assess queen production is feasible, how high is the natural variation in queen numbers and queen weight/size? The test design followed the ICPPR working group semi-field test protocol with *Phacelia tanacetifolia* as a crop. In the period before the hives reached the "switch-point" they were closely monitored and relevant parameters, i.e. colony weight and occurrence of queen larvae, were noted. To prevent young queens from leaving the hives queen excluder were installed at the hive entrances. After young queens had hatched they were collected from the hives to avert overcrowding and associated food shortage in the hives. From the results it can be concluded that hive weight was not a good predictor of the "switch-point", whereas monitoring of the occurrence of queen larvae in the hives was a more reliable method. Concerning the use of queen excluders and collection of young queens before the final brood assessment, this new approach yielded a sufficiently high number of young queens in every replicate to determine reliable mean values for queen weight/size.

TU199

First experiences and first steps towards a a chronic bumble bee (Bombus terrestris) test design

S. Kimmel, Innovative Environmental Services (IES) Ltd. / Ecotoxicology; M. Cornement, Innovative Environmental Services IES Ltd; T. Schmidt, IES Ltd / Ecotoxicology; S. Höger, Innovative Environmental Services (IES) Ltd / Environmental Toxicology

With the European Food Safety Authority (EFSA) Guidance Document on the risk assessment of plant protection products on bees (EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295), a number of changes and impacts on assessing the potential risk for pollinators has moved into focus. While some of the newly requested test setups and endpoints have undergone development and in some cases multiple ringtest phases, other designs still phase lack of data or time to develop the methodology properly. The here presented approach is intended to help on the way forward in fulfilling the multiple amount of new requests within the above stated document. Results for controls and reference item treatments from several data sets are presented. Recommendations on possible modification of the test design are discussed. Main goal of the presented data is to move forward with chronic bumble bee testing towards a robust, feasible and reliable test design / guideline proposal useful for additional risk assessment data needs and calculations. Based on the presented data, a setup of an international ringtest phase may be accommodated. \n

TU200

On the way to a new guideline - Results of three years of bumble bee semi-field testing

A. Alscher, S. Hecht-Rost, J. Lueckmann, Rifcon GmbH

Up to now, no official guideline for standardised semi-field trials exists to assess effects of pesticides on bumble bees. Open questions are: What are relevant endpoints for bumble bees? How can these endpoints be assessed in a reliable, reproducible way? To answer these questions, tests have been performed from 2014 to 2016 based on the recommendations of the ICPPR Non-*Apis* workgroup of 2015 and 2016. Experiences were gained regarding different crops, season and size of colonies. Following endpoints were assessed: brood development, mortality, flight and foraging activity, colony mass and reproduction (young queens and drones). Selected results will be shown and advantages/shortcomings of different designs and endpoints discussed. \n

TU201

Experimental design for semi-field trials with solitary bees: Status quo after three years of development

L. Franke, J. Fricke, Eurofins Agroscience Services Ecotox GmbH / Ecotoxicology Field; O. Klein, Eurofins Agroscience Services EcoChem GmbH / Ecotox Field; S. Knaebe, EAS Ecotox GmbH / Ecotox Field

The EFSA guidance document on the risk assessment of plant protection products for pollinators published in 2013 stated that data for bee species other than honeybees are needed. To close this data gap it was proposed to develop study designs for non-Apis bee species. Based on existing protocols for honey bee studies (OEPP/EPPO Guideline No. 170) and suggestions from meetings of the ICPPR non-Apis workgroup in 2015 and 2016 a semi-field test design for solitary bees was developed. First semi-field tests with the red mason bee (*Osmia bicornis* L; Hymenoptera, Megachilidae) were conducted in 2014 with different set-ups and release techniques to develop a method with high hatching success and good establishment of females at the nesting units. Based on the most promising experimental methodology reference substances were tested in 2015 and 2016. The references were dimethoate, which affects adult bees, and fenoxycarb, a brood-affecting insect growth regulator. The following set-up for a semi-field test with solitary bees is proposed: One nesting unit with 100 nesting cavities is placed in each tunnel. 60 females and 90 males are released. The adult bees and their progeny are exposed to the nectar and pollen of the treated crop throughout the whole flowering period. After the end of the exposure phase brood development is followed until hatching of the progeny next spring. The following endpoints have to be observed in the studies: nest occupation of females, flight activity at the nesting units, reproduction capacity and hatching success of the progeny. Concerning the tested reference items *O. bicornis* was very sensitive to dimethoate at rates well below the rate used in semi-field honey bee trials. However, the results for fenoxycarb were inconsistent. Based on the available results it is recommended to test alternative brood affecting substances. In summary the developed experimental design and the selected endpoints are appropriate to detect effects from pesticides on the solitary bee species *O. bicornis*. Additionally, the statistical analysis proofed that the experimental design is valid and repeatable. However, some open questions still remain and need further research, e.g. how fit are solitary bees out of their natural season (in summer) and which substance can be used as a reference item for brood studies?

TU202

Fenoxycarb, a suitable reference item in semi-field testing on the solitary bee Osmia bicornis (Hymenoptera, Megachilidae)?

J. Lueckmann, C. Classen, Rifcon GmbH

According to the EFSA Guidance Document on the risk assessment of plant protection products on bees, not only honeybees but also bumble bees and solitary bees have to be considered. But for testing of solitary bees under laboratory, semi-field and field conditions, no official test guidelines exist. Regarding the semi-field exposure a design for *Osmia cornuta* and *O. bicornis* was developed, which is based on the EFSA document, testing principles for semi-field studies (EPPO 2010) and the outcome of an ICPPR non-*Apis* workshop in 2015. Based on practical experiences the design was adapted on a second workshop in 2016. Next to others the number of cells produced per female and the non-successful development of such cells, expressed as the 'brood termination rate' (BTR) were regarded as key endpoints. One result of a study from 2015 performed with *O. bicornis* was that the application rates of the test item fenoxycarb (150 and 350 g a.s./ha), an IGR routinely used in bee brood studies as a reference item (OECD 2007), produced statistically significant increased BTRs (Knäbe et al. 2016). However, they were below 50% for both rates which has to be regarded as not being adequate for a reference item. Therefore, a semi-field study performed in 2016 investigated the effects of a higher dose. This study consisted of a control and fenoxycarb treatment group, each with 4 replicates, *i.e.* tunnels with 90 m^2 of flowering *Phacelia*. Nesting units with cocoons of both sexes of *O. bicornis* were set-up 10 days before application to let the bees hatch, mate and to establish females in nesting activity. Fenoxycarb was applied at a rate of 600 g a.s./ha (four times the approved single rate) during flight activity. Bees were exposed to the treated crop for 10 days. Flight activity was assessed on the day of application and the day after. The number of nesting females and the number of cells produced was determined every 2nd or 3rd day after application. The subsequent brood development was investigated at a monitoring site. Finally, the number of cells and cocoons produced per female was assessed and the BTR for the control and test item was determined. In the current poster the results of the study are presented which show that the proposed test design is suitable to perform semi-field studies on *O. bicornis* in *Phacelia*. Finally, the question is discussed whether fenoxycarb is a suitable reference item for semi-field studies on *O. bicornis.*

TU203

Analysis of the effects of thiamethoxam in the brain of africanized honey bees through MALDI-imaging technique

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Africanized bees *Apis mellifera* are important for pollination and food production. The search for the increasing of agricultural production has led to expansion of cultivated areas and a greater dependence on pesticide use. This can affect non-target insects such as bees. Among most widely used insecticide classes in agricultural crops there are the neonicotinoids. As a member of this class, there is thiamethoxan, an neurotoxic insecticide widely used in Brazil. Based on the importance of toxicological studies on the evaluation of possible harmful effects that pesticides can cause on bees, this study aimed to evaluate the effects of exposure to a sublethal concentration of thiamethoxam on the distribution of specific proteins identified in the brain of *Apis mellifera*, through MALDI Mass Spectrometric Imaging (MALDI-MSI) technique. For this purpose, forager bees were exposed to an 8-day diet containing a sublethal concentration of thiamethoxam corresponding to $LC_{50/100}$. The individuals were collected on the 1st and 8th days after the beginning of food supply, and the brains were sectioned in a cryostat. A chemical printer ChIP-1000 (Shimadzu) was used for matrix and trypsin deposition, and spectra were acquired using a MALDI-TOF-TOF (Shimadzu). The spectra were converted into images by MSiReader v0.05 software, thus generating density maps for proteins. Glutamine Synthetase (GS) and Thioredoxin Peroxidase I (TPxI) protect tissues against oxidative stress. Both proteins had their relative intensity of expression increased in the exposed groups comparing to the control groups. The enzyme GS has a function of protecting neurons from toxicity by controlling excess ammonia and glutamate, converting them to glutamine. The increased intensity of the expression of this enzyme in specific areas of the brain reflects an attempt to balance the increase of ammonia and glutamate caused by exposure to the insecticide, protecting the neurons against neuronal degeneration. A large number of cellular functions are based on thioredoxin, as the cellular response to oxidative stress. Thus, the increased relative expression intensity of this protein in the groups exposed to thiamethoxam, shows an attempt to detoxify the organism against reactive oxygen species caused by such exposure. The alteration in the relative expression of these proteins demonstrate that imidacloprid could cause biochemical alterations that can imbalance the neuronal functions.

TU205

Linking protection goals to trigger values using compound specific properties: Chronic risks to bees

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In the EFSA guidance document for the assessment of risk of plant protection products (PPP) to bees a number of new trigger values are proposed. One of concern due to its conservative nature is the honey bee chronic oral trigger of 0.03. In effect a substance is considered low risk if the LDD_{50} is 34x higher than the estimated exposure. An impact analysis indicated that using this trigger almost all substances would not pass the screening or tier I risk assessment leading to higher tier evaluations even for substances of low toxicity. As the risk assessment for a single stressor (PPP) is over conservative it will make the assessment of risk due to multiple stressors meaningless. We analysed EFSA's methods and the underlying assumptions to calculate the actual level of protection afforded by this trigger to a range of PPP. In almost every case the level of protection achieved greatly exceeded the specific protection goal (SPG) < 7%. Mathematically, the trigger value of 0.03 only meets the SPG for a given LDD_{50} if the slope (b) of the dose-response relationship is exactly 1.43. If the slope is greater the level of protection will exceed the SPG which is the case for the majority of compounds and generates a large number of false positives. For many other substances the measurement of a LDD_{50} is not technically possible due to low toxicity and/or limited solubility (e.g. many herbicides and fungicides). In these cases only a no observable effect daily dose (NOEDD) can be determined. The use of a NOEDD also leads to an exceedance of the level of protection and false positives (i.e. low risk is indicated at $^1/_{34}$ of the NOEDD). We present a simple method to evaluate all PPP to the same level of protection by taking into account the type of endpoint (i.e. LDD_{50} or NOEDD) and the slope of the dose response relationship. In addition the actual level of protection afforded by a given exposure toxicity ratio (ETR) can be calculated allowing for better informed decision making by risk managers. The number of false positive and negatives in a risk assessment could be reduced by using specific triggers based on the properties of the test substance. In complex models such as BEEHAVE (Becher et al., 2104) which have the potential to assess both single PPP stressors and interactions with multiple stressors (e.g. disease and Varroa mites) the type of endpoint and the shape and slope of the dose-response curve need to be taken into consideration.

TU206
Improving pesticide regulation by use of impact analyses: A case study for bees
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When changes to regulatory guidance for risk assessment are proposed it is necessary to undertake an analysis to assess whether they bring the desired improvement to a risk assessment and reliability of the outcomes to inform decision making. In particular, impact analysis should estimate the chances of getting both false negative (concluding low risk where more research is needed) and false positive outcomes (concluding high risks where the product is of low risk). Such analyses are used to inform on future product development costs, reliability and efficiency of the assessment, and workload for regulatory authorities. In this poster presentation, we present the findings from an impact analysis conducted on the proposed EFSA bee guidance document (2013) and discuss whether the proposed guidance would provide a reliable and efficient tiered approach toward the protection of bees due to the potential risks posed by the use of plant protection products (PPP). Following on from this, a second impact assessment is presented based on new data generated by ECPA member companies regarding the assessment of chronic risk to bees. Critical areas are discussed and suggestions for improvement of the risk assessment for PPP to bees are presented. To be reliable, impact analyses require large quantities of data. In the context of our analysis the studies were performed to meet regulatory requirements and follow standardized test guidelines. At present this type of data are of limited interest for publication as they are typically not considered to be original research.

TU207
Risk mitigation measures for the protection of flower visiting insects from effects of pesticides
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Latest research on biodiversity in agricultural landscapes suggests that flower visiting insects (FVI) such as wild bees and butterflies are not sufficiently protected from the effects of pesticides. To counteract this situation, the implementation of appropriate risk mitigation measures might be useful. Within the research and development project "Protection of wild pollinators in pesticide risk assessment and management" (FKZ: 3715 64 409 0; funded by the German Federal Environment Agency) recommendations for risk mitigation measures which might be suitable to protect FVIs were developed. Based on current available literature these measures were evaluated (1) according to their efficacy to reduce exposure of FVIs in off-field habitats by a reduction of pesticide entries in off-field non target areas, (2) according to their efficacy to reduce the exposure of FVIs in-field, and (3) according to their potential to reduce pesticide effects on populations of FVIs by the improvement of habitats (e.g. via providing foraging habitats). Moreover, acceptability and feasibility for implementation were characterized. Most important results to selected risk mitigation measures (e.g. in-field buffer strips) are presented in the poster.

Advancements in life cycle impact assessment and footprint method development (P)

TU208
Including freshwater in the Area of Protection Resources
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Freshwater as a resource has a very particular status in LCA modeling. It is a vital resource in sustaining ecosystems and human health, while, unlike other resources, there is no substitute for certain functions, and both consumption (source function) and pollution (sink function) can affect it. Up to now, two methods link freshwater consumption to the Area of Protection (AoP) natural resources. Both reflect a quantitative change in long-term availability. However, the quality of the freshwater resources remaining for future users is also important. Today, water degradation due to emissions is considered as impacts on ecosystem quality and human health (e.g. toxicity or eutrophication), but no method links water pollution (i.e. quality) to the AoP natural resources. It is not obvious how to distinguish between the damage of an emission on ecosystems/human health and the damage on the resource itself without double counting impacts. This study provides insight into how to consider freshwater resources as an entity to protect within the AoP natural resources. The objectives are to (1) frame a definition for water as a resource avoiding overlaps with human health and ecosystem impacts, (2) describe the impact pathways affecting this resource, (3) propose a characterization framework to evaluate the character of freshwater as a life-supporting resource. The key element in the definition is that freshwater resources are those remaining for future generations, or for the future uses of the present generation. Three pathways have been identified that may impact freshwater natural resources: (1) the fraction of water pollution not taken in by humans or ecosystems, (2) the persistence of pollutants in water or adjacent compartments resulting in its continuous pollution, and (3) water quality improvements via water importation or treatment, which improve water quality above its quality level during abstraction. Current indicators do not allow considering the environmental benefit of increasing resource availability and, most notably its quality, for future uses, while a freshwater resource indicator would be a relevant metric to capture that. Therefore, such an indicator would allow LCIA to capture impacts that cannot be covered by a human health or an ecosystem quality indicator. It seems essential to reflect those issues in the LCA results in order to transparently advise decision makers about potential safe water supply in the future.

TU209
LCA-based green water footprint
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Precipitation, including both green and blue water components is a key resource for the diversity and resilience of aquatic and terrestrial ecosystems, as well as for sustainable development. A comparison of different models allowed to identify that green water use in global crop production is about 4–5 times greater than the consumptive use of blue water. Green water use refers to precipitation on land that does not run off or recharge the groundwater but is stored in the soil or temporarily stays on top of the soil or vegetation. It also refers to the rainwater incorporated into harvested crops or wood. While the consideration of blue water use in Life Cycle Assessment (LCA) has been rapidly evolved in the last years, the development of methods for addressing the green water flows and develop green water scarcity indicators have been progressing much more slowly. Green water flows refer to the portions of green water used by soil and vegetation that is evaporated and transpired (ET). The ET is the sum of vapour water flows from soil evaporation, wet canopy evaporation and plant transpiration at dry canopy surface that returns to the atmosphere. This paper presents an LCA-based green water footprint to model the impacts of green water flows considering two different mechanisms: (1) potential disturbances of regional long-term availability of surface blue water (called as green water and soil interface - WS) due to changes in green water use, and; (2) potential perturbations in the ET that is supplied to the atmosphere, and then, in the portion of precipitation that returns to the same basin (called as green water and atmosphere interface - WA), due to land use.Therefore, this study is devoted to the development of mid-point indicators of green water scarcity at 30-arcmin global grid, for both interfaces identified. Green water scarcity indicators at both interfaces were, then aggregated to single global weighted average green water scarcity indicators at the same resolution, indicating the regional variability of green water scarcity, CF_{green}. The presented life cycle impact assessment method for assessing the green water footprint is useful for environmental decision-support in the production of rain feed agriculture and forestry, allowing to accounting for green water scarcity in decision-maker's environmental calculations in a consistent and acceptable manner, having in mind the available blue water resources after ecosystem and human demand.

TU210
The Challenges of Global Surface and Groundwater Scarcity
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Water scarcity poses major challenges for humanity and ecosystems. Since surface and groundwater differ both hydrologically and in how they satisfy socio-economic and ecological demands, it is important to differentiate between the two water resources. In this study, we calculated water scarcity indices (ranging from 0 [low] to 1 [high]) at global scale for both water resources in 1981-1990 and in 2001-2010. Additionally, we increase the spatial resolution from watershed level to 0.5 degrees to better account for local scarcity. Using a multi-model ensemble enabled us to assess uncertainties based on latin hypercube sampling and Monte Carlo simulations. An increase in water scarcity was, furthermore, examined with two statistical significance tests: the Wilcoxon–Mann–Whitney test and the Mann–Kendall trend test. Globally, water scarcity amounts to 0.51 in 2001-2010. This indicates that we have already crossed the boundary between moderate and severe water scarcity (WSI > 0.5). The Monte Carlo simulations reveal that it applies with

a likelihood of 56%, while it is 100% certain that we are at least living under moderate water scarcity (WSI > 0.1) at the global level. Groundwater scarcity is more pronounced than surface water scarcity and concerns more extensive regions. It suggests to optimize water use for its source. However, higher stress in groundwater consumption might be due to the water allocation procedure where preference is given to the use of groundwater. Additionally, groundwater is more difficult to model, as calibration and validation data are much scarcer for groundwater than for surface water. The high groundwater scarcity and high uncertainties both highlight the need for further research in groundwater resources and assessment of its impacts. Our results indicate that water scarcity slightly increased from 1981–1990 to 2001–2010, although its likelihood is only 53%. The Wilcoxon–Mann–Whitney test confirms that the decadal average increased (p-value < 0.01). However, the Mann–Kendall trend test shows that there is no statistical evidence for an increasing trend (p-value = 0.19). Although a trend is not evident, the current situation clearly demonstrates the urgency to develop strategies to alleviate water scarcity. With our results, differences in ground- and surface water consumption can be assessed in water scarcity footprints and the robustness of results can be evaluated through uncertainty analysis.

TU211
ReCiPe2016: a harmonized life cycle impact assessment method at midpoint and endpoint level
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To further progress LCIA methods beyond the current consensus state of the art, we updated the ReCiPe2008 method to its version of 2016. ReCiPe provides a harmonized implementation of cause-effect pathways for the calculation of both midpoint and endpoint characterisation factors. In order to make a step forward in overcoming current shortcomings, the update of ReCiPe focused on (1) providing characterisation factors that are representative for the global scale, while maintaining the possibility for a number of impact categories to implement characterisation factors at a country and continental scale and (2) improving the methods applied to model midpoint to endpoint factors. We implemented human health, ecosystem quality and resource scarcity as three areas of protection. Endpoint characterisation factors, directly related to the areas of protection, were directly derived from midpoint characterisation factors with a constant mid-to-endpoint-factor per impact category. We included 17 midpoint impact categories. Compared to ReCiPe2008, impacts of water use on human health, freshwater ecosystems, and terrestrial ecosystems; impacts of climate change on freshwater ecosystems; and impacts of tropospheric ozone formation on terrestrial ecosystems were added in ReCiPe2016. Comparing the mid- to endpoint factors of the new and old ReCiPe, it can be seen that the improvements of the methodology lead to substantial differences for several impact categories. To show the implications of these differences, a case study is performed in which the life cycle impacts of a number of food consumption items will be determined with the old and the new version of ReCiPe. Although significant effort has been put into the update of ReCiPe, there is still major improvement potential in the way impact pathways are modelled. Further improvements relate to a regionalisation of more impact categories, and adding more impact pathways.

TU212
Assessing impacts of land use change using predictive, spatial modelling
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The agricultural expansion and intensification required to meet growing food and agri-based product demand presents important challenges to future levels and management of biodiversity and ecosystem services. Recent developments in life cycle assessment (LCA) allow for some consideration of the relationship between land use and ecosystem services, but limitations remain. For example, methods for estimating land use change (LUC) tend to be based on historical changes at the country level; these may not be indicative of potential future changes. In addition, the impacts of such changes are computed on a per area basis. Spatial heterogeneity, a key factor in determining impacts on biodiversity and many ecosystem services, is often ignored. Our approach involves the integration of land change modelling (LCM) and ecosystem services modelling into LCA in a Land Use Change Improved (LUCI)-LCA. This is done by substituting key elements of life cycle inventory with the outputs of the aforementioned modelling. Land change modelling is achieved using logistic regression techniques based on suitability of land for agricultural production, or scenarios generated on the basis of proximity to existing agriculture. Both approaches account for intensification and expansion responses to agri-based product demand. Ecosystem impacts (greenhouse gas emissions, water consumption, erosion potential, nutrient leaching and biodiversity loss) associated with predicted land use changes are derived using the newly updated InVEST software suite. The method utilises public, globally available spatial data. We apply our approach to explore the implications of large-scale growth in demand for bio-based, high-density polyethylene (HDPE). Several production volume scenarios are considered, representing the types of changes that could arise from company or sector-level shifts in demand. Two alternative feedstock-location combinations are assessed. Model predictions of land use change were cross-validated using historical data, pointing to much better accuracy of LUCI-LCA methods as compared to standard approaches used in attributional LCA. The approach can be used to guide future policy, innovation and sourcing decisions, facilitating evaluation of impacts of different bio-based technologies and the potential non-linear impacts of scaling such technologies. Identification of locations with lower impacts is also possible.

TU213
Developing global characterisation factors for habitat fragmentation in LCIA
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Alongside climate change or pollution, habitat change and fragmentation was identified by the Millennium Ecosystem Assessment as one of the main direct drivers of biodiversity loss. Given this fact, it seems to be of adequate importance to include this driver in LCIA. Nevertheless, while habitat loss is already implemented in LCIA methods, the additional impact on biodiversity due to fragmentation is not yet assessed. In line with this, the UNEP-SETAC land-use group recently pointed out that habitat composition and configuration, including fragmentation, seemed to be under-represented in impact assessment models. A literature review in ecology showed that among elaborated indicators linking the landscape morphological attributes with species biological characteristics, the metapopulation capacity λ_{self} can be used to rank different fragmented landscapes in terms of their capacity to support viable metapopulations. This work proposes to adopt this indicator to consider fragmentation in LCIA. First, we developed a routine procedure to calculate λ_{self} for 100 km²-landscapes. Second, we tested the sensitivity of the procedure to selected parameters and derived λ_{self} for a whole ecoregion. Finally, λ_{self} was computed for all forest ecoregions included in the 36 Biodiversity Hotspots. The results highlighted a significant difference between ecoregions, showing that fragmentation should be included in LCIA methods. Thus, based on the values of λ_{self} derived for more than 300 ecoregions all around the world, it is proposed to compute a Fragmentation Index (FI) at a global scale ranging from 0 (low stress) to 1 (high stress). FI can then be adopted as a midpoint Characterisation Factor (CF) in LCIA to weigh the use of 1 m² of land in highly fragmented landscapes. Various options to compute FI are discussed along with the scale for a practical use in LCIA (e.g., biodiversity hotspot, ecoregion, country). The next step will be to derive an endpoint CF for the impact of not only habitat loss but also fragmentation by using the SFAR model (Species Fragmented-Area Relationship) based on λ, and to compare it with current endpoint CFs. In conclusion, this work aimed at developing and operationalising a large-scale calculation of one of the most comprehensive ecological indicators for fragmentation and proposing a global Fragmentation Index for all forest ecoregions affected by fragmentation. Work is still needed to fully operationalise its use in LCIA.

TU214
Framing the characterization of life cycle impacts of land use on ecosystem services: an integrated consequential approach
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The characterization of land use impacts on ecosystem services in life cycle impact assessment has not reached consensus yet and is hence not applied in current LCA practices. To support the development of models, for both the life cycle inventory of land use and the assessment of impacts on ecosystems, a new framework is presented here. Proposing an alternative to current models (with which it still shares common aspects), it follows a consequential life cycle approach, which allows to take an actual reference situation when modelling land use changes and ecosystem services. Thanks to this, an integrated valuation of ecosystem benefits to society's well-being is possible, while keeping information on the ecological impact of land use changes interventions.\nIn details, the advanced methodological framework first promotes the use of dynamic predictive land use changes models to complement the information included in current land use inventories. These usually

lack a consistent quantification of land cover and use changes, and are regionalized at a too low scale to be useful when assessing impacts on ecosystem services, which happen at a local scale. To test the approach, an integrated land use model is implemented over Luxembourg and results of land use changes inventorying are presented.\nIn a second time, the development of associated characterization models to calculate midpoint and endpoint indicators of land use changes impacts on ecosystem services is framed. A consequential cause-effect chain is depicted, and the integrated valuation of ecosystem benefits, which is done by coupling socio-economic models to ecological models, is promoted. Changes in the provision of services (focusing particularly on the services of pollination and carbon sequestration) are calculated by implementing an integrated MIMES model in a spatialized system dynamics environment. These results are then interpreted to obtain characterization factors applicable to the previously defined land use changes flows. The ecosystem services indicators used are bio-physically quantified at midpoint level and monetized at endpoint level.\nNext to presenting and testing the framework, its validity, potential complexity and practical application are also discussed. These aspects must be treated when developing increasingly complex models that tackle broader and more intricate issues of global changes, the results of which have to be credible in a context of decision-making.

TU215

A new method to integrate noise impact from road mobility in life cycle assessment - preliminary results.

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WHO (2011) reported very comprehensively on the burden of disease from environmental noise, highlighting noise as a threat to public health. As shown in Meyer et al. (2016), there are still significant variations and shortcomings between methods aiming to include environmental noise damage on human health in life cycle assessment (LCA) and none of them has become consensual so far. A new approach is proposed here, considering the main strengths of the published methods and including noise modelling according to the best practices in the acoustics research community. To include noise in LCA, the marginal impact of an additional unit of traffic has to be known. This is achieved by running two successive simulations, with and without considering a proportional increase of the studied traffic. By comparing the results of these two simulations, a change in human health damage is obtained that can be further linked to the additional traffic. After normalization, a characterization factor (CF) in DALYs by vkm is obtained for the geographical area considered. This operation can be repeated in different geographical areas, for different health impairments and for different time periods to study how this CF is dependent of these different parameters. In a first application of this methodology, data relative to the area of Lyon (France) were used. This large territory have been cut in homogeneous parts. More than seventy of these geographical areas have been processed for light vehicles to study the variability of the resulting CF and identify a potential typology if the variation is significant. The average CFs for annoyance and sleep disturbance, 1.32E-07 and 2.04E-06 DALYs/vkm, are coherent with literature data. The ratio minimum to maximum shows that the CFs vary of approximately two orders of magnitude. However, the distribution of the results is highly asymmetrical on a linear scale and seems to be well fitted by a lognormal distribution. With the shape of the distribution and the relatively low variability of the outputs, identifying a typology is difficult. The final CFs built with this method should be more accurate than existent ones and, at the end, should allow systematic integration of noise impact in LCA. Moreover, doing multiple calculations in different locations will lead to a quantification of the variability of this impact and thus an uncertainty distribution could be proposed for the final CFs, increasing their usefulness.

TU216

How Sustainable is Hydropower Really?

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Hydropower is an important renewable energy source, but it can adversely affect water scarcity and climate change. Improved water and carbon footprints of nations' hydropower production are needed to better understand its environmental impacts, since hydropower production greatly varies in site-specific impacts, as against most power production technologies. We determined both footprints for the year 2009 for ~1500 globally distributed hydropower plants, covering >40% of global annual electricity generation. Since a reservoir can fulfil multiple purposes, impacts were allocated to hydropower based on its ranking among all the purposes the reservoir fulfils. National averages were weighted by the dams' annual electricity generation in 2009. Water footprints were calculated applying a monthly water balance approach to the associated reservoirs. The monthly water consumption was multiplied with monthly water scarcity indices and allows accounting for benefits of storing water in wet seasons, while releasing it in dry seasons. In addition, the impacts of flow alterations were assessed, reflecting that reduced as well as increased river discharges can damage ecosystems. Carbon footprints were estimated by generalized linear modelling. About 100 records were available as training dataset. Explanatory variables were selected based on multi-model inference and include the reservoir area, the area-to-electricity ratio, the age, the maximum temperature and the erosion rate. While previous studies mostly overrated the impacts from hydropower's water consumption, they underestimated its climate change impacts. Water footprints can even be negative, which indicates that the reservoirs alleviate water scarcity. On the other hand, flow alterations often cause higher adverse impacts on the environment than water consumption does. With regards to carbon footprints, CO_2 emissions contribute more to the global warming potential of hydropower than methane emissions do. The production-weighted water footprint of the analysed hydropower plants amounts to 55 m^3 H_2O_e / GJ, and the carbon footprint is 76 g CO_{2e} / MJ. However, both vary considerably across nations and even more on individual plant level. China as the largest hydroelectricity generator has water and carbon footprints below the global average. Overall, the results cast doubt on the environmental sustainability of hydropower, and each new hydropower project should be assessed carefully.

TU217

Advances in integrating the hydrological cycle in a multimedia assessment framework for LCIA of water consumption

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A recent analysis of life cycle impact assessment (LCIA) methods adressing impacts of water consumption on ecosystems showed that available methods cannot or only partially be compared and combined within a consistent framework (Núñez et al., 2016). To overcome such compatibility problems, the Water Use in LCA (WULCA) working group tackles this issue within the UNEP-SETAC life cycle initiative, and is currently developing a comprehensive framework that should result in consensus-based guidelines. Some distinctive attributes of the framework include the following: i. A mechanistic model, meaning that the indicators are based on a sequence of interconnected sub-factors in the characterisation factor. These sub-factors link human activities (i.e., life cycle inventory, LCI) to potential midpoint and endpoint impacts on ecosystem quality. Generic water scarcity indices are not part of the equation, since they are not part of the cause effect chain addressing potential environmental damage (Boulay et al., 2014) ii. A multimedia model that represents the hydrological cycle based on two types of elements: water storage compartments (stocks) and connections between compartments (flows). This approach will allow one to differentiate impacts on ecosystems from consuming water from different sources. Moreover, it will render the assessment of land use-related impacts on the hydrological balance and water consumption impacts fully compatible. In other words, we will no longer need to distinguish between green water (soil water) and blue water (river and aquifer water) consumption and impacts, as both water flows are regarded in the multimedia model. This has largely been a subject of discussion in LCA and water footprinting, since there is no clear separation of green and blue water iii. A flexible structure organised in matrices that can be regarded by both midpoint and endpoint methods. This structure will facilitate, among others, comparison between intermediate results (i.e., for one specific sub-factor of the characterisation factor), easy update with e.g., new water compartments, and tracking exchanges between different compartments, which also avoids double counting of impacts. This poster will explain the main framework's properties and focus on the results of the fate modelling. It will discuss how its implementation will improve the environmental relevance of and compatibility between LCA water consumption indicators.

TU218

Water footprint assessment of wine production in Galicia (NW Spain)

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Water stress is becoming a major environmental concern so that the development of tools for improving the performance management and quality of this resource is a primary consideration among the scientific community. Life cycle assessment (LCA), in particular the water footprint assessment, is one of these tools. Traditionally, wine industry has considered that wine production consumes from 3 to 6 liters of water per liter of wine during winemaking process. Nonetheless, upstream processes (viticulture) and indirect water consumption are traditionally disregarded. The present study analyses the water footprint of one bottle of withe wine (750 mL) from a cradle to gate approach (i.e. from grape production to the production of bottled wine at winery gate ready to dispatch). Hence, a Galician medium-size winery in the *Rías Baixas* appellation was assessed during year 2015. Guidelines and requirements of ISO 14046: 2014 standard on water footprint were followed to carry out water-related performance, taking into account both degradative and consumptive water use. Results of the study confirmed that ISO methodology provides consistent outcomes in terms of hotspots and water efficiency and management, as well as is useful for supporting decision-making by the wine sector. Acknowledgements Dr. Pedro Villanueva-Rey would like to thank the Galician Government for financial support (I2C postdoctoral student grants programme).

TU219

A new generation of characterization factors for the life cycle impact assessment of land use on ecosystem services

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Ecosystem services (ES) are beneficial inputs to the well-being of people and also drivers of technological development. The value of (or pressure on the supply of) ES in Life Cycle Impact Assessment (LCIA) is usually measured by considering indicators of potential production for some ‘provisional’ and ‘regulating and maintenance’ services. Despite some valuable advances, the end-point indicators of land use impacts on natural resources and ecosystem health still do not evaluate potential costs to society in terms of ES. Approaches to assess land use effects on ecosystems’ functionality and its relation to ES supply hence remain fragmented in LCIA. The aim here is to present final results of VALUES (*http://www.lifecycle-values.lu/*), a Luxembourg National Research Fund’s project initiated in 2014 to develop a new generation of characterization factors (CFs) to assess land use-driven impacts on ES. The LCIA method formulated in VALUES is implemented with a system dynamics (SD) rationale, where CFs are calculated as marginal changes in the contribution of ES to human well-being and production indicators, e.g. the additional monetary cost (or benefit) that would arise in term of ES supply from increasing a land use type by one unit over a certain time and space. To this end, an integrated model is built upon an environmentally extended multi-regional input-output database coupled to ecological process and land cover/use models within an existing dynamic multi-scale ecological-economic framework. This modelling framework allows to forecast trade-offs and synergies among ES values, capturing cross-linkages and feedbacks occurring along the cause-effect chain from inventory flows to areas of protection. By running simulations according to different global to regional socio-economic and land use scenarios, marginal changes in monetary values of ES per regionalized land use unit areas are computed, which can afterwards be translated into CFs. Accordingly, VALUES provides a novel operational modelling framework for LCIA able to retrieve spatially- and temporally-explicit CFs for ES with a multi-regional and integrated scope. Archetypal sets of CFs for different ES (e.g. C seq./storage, pollination, water supply/regulation, soil formation, forest products, aesthetic/recreation potential, etc.) are under calculation, and will be downloadable in spreadsheet format for direct use in life cycle assessment tools after the end of the project (March 2017).

TU220

Harmonizing the assessment of resource use in LCA - First results of the task force on natural resources of the UNEP-SETAC global guidance on environmental life cycle impact assessment project

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(Mineral) natural resources are of great relevance for industry and society. However, consensus on how the use of bauxite, iron ore, silica sand, etc. should be considered in Life Cycle Impact Assessment (LCIA) is currently lacking. While environmental impacts from mining and refining are captured in various impact categories (e.g. acidification or toxicity and related impacts on human health and ecosystem quality), the assessment of resource use as such is still debated. Resource depletion impact categories (e.g. depletion of metals and minerals or of fossils) are still not well defined and numerous methods and models exist. Therefore, within the UNEP-SETAC Life Cycle Initiative’s flagship project on providing guidance on environmental LCIA indicators, a new task force on natural resources in LCIA was launched in the end of 2016. The task force, which focuses on mineral primary resources and comprises experts from academia, the mining industry, OEMs and other stakeholders, aims at finding a consensus on the assessment of resource use in LCIA. First, the scope of the assessment, which can range from geologic depletion, anthropogenic availability, energy and exergy consumption, socio-economic availability, until social acceptance will be defined. This process includes the evaluation of existing methods and consensus finding on terminology as well as on data choices. Furthermore, the task force intends to develop a consensus characterization model for assessing impacts of (mineral) resource use. It is an open question whether this method development will be based on (1) enhancing/refining an existing impact assessment method or (2) merging existing methods addressing various relevant aspects or (3) a newly developed approach. This poster presentation will show first results of the international and interdisciplinary consensus finding process and provide an opportunity to share and discuss the work in progress with the LCA community and other interested parties, e.g. from the mining and metal industries.

TU221

Making biotic resources count in the LCIA framework

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Natural resources provided by the Earth, both biotic and abiotic (i.e. raw materials, energy, water, air, land and soil as well as biodiversity and ecosystems) represent crucial economy and life-support elements for human societies worldwide. Indeed, natural resources are a building block in the supply chain, thus pushing the economic growth, and provide global functions, as in climate regulation. However, in a globalized world where population is in continuous expansion and the demand for finite resources continues to rocket, the current production and consumption patterns in developed and developing countries are generating concerns about their sustainability, with particular regard to the potential repercussion on the environment and climate. On such a background, a transition towards a bio-based economy, called “bio-economy”, is necessary, by improving the efficiency in resource use for both abiotic and biotic resources, in order to meet the challenging objectives included in the Sustainable Development Goals. This transition requires a solid model of impact assessment to be developed, especially for natural occurring biotic resources, as their sustainable use should be ensured. Over the last decades, a handful of methodologies, including life cycle oriented approaches for biotic resource depletion, have been developed with different purposes. Nevertheless, within supply chain management, a robust and comprehensive characterization of biotic resources and a reliable set of indicators are missing. Therefore, this study aims at providing key elements of the ongoing discussion on the environmental and socio-economic relevance of biotic resources, by on one hand improving their accounting for supply chain assessment and on the other hand defining an impact assessment model based on renewal times. As a result, a list of natural biotic resources and their availability worldwide was drawn, based on data from the existing literature, in order to cover the conceptual gap of elementary flows associated to them within the inventories of the LCA framework. Then, on this basis, suggestions based on renewability concepts were made for identifying reliable indicators to evaluate the depletion of biotic resources in the LCA context and support the transition to sustainable bio-economy at global scale.

TU222

Soil carbon stock change after digestate application

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One strategy to reduce greenhouse gas (GHG) – emissions in Germany has been the development, use and promotion of renewable energy systems. In Germany biogas production has been growing over the past decades. Currently around 8000 biogas plants operate with an installed capacity close to 4 GW_{el}. The residue from biogas production, sometimes called digestate, is returned to agricultural fields in order to recycle nutrients and organic carbon. Whether the soil carbon stock increases or decreases after digestate application is controversially discussed. Carbon dioxide and methane are measured on the field after the application of digestate at five locations in Germany. The change of the soil carbon stock is estimated by using the net CO_2 and CH_4 exchange rates in combination with C-input from digestate and C-uptake by the harvested crop. Preliminary estimations indicate that the carbon content in the soil can change substantially after digestate application. The soil carbon change after digestate application can alter the greenhouse gas balance of biogas systems depending on site-specific factors. The transfer of nutrients in crop rotations and the change in soil carbon are still major challenges in life cycle assessment of agricultural systems.

TU223

The development and application of a new characterisation method for enhanced exposure to Naturally Occurring Radioactive Materials (NORM)

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Nearly 70% of human exposure to ionising radiation results from Naturally Occurring Radioactive Materials (NORM). Exposure to naturally occurring radionuclides is part of daily life, indeed they form a part of the rocks and soils we live our lives on, and the food we eat. The resulting radiation is a part of what it termed ‘background radiation’. However, a number of common industrial processes and applications have the potential to expose both humans and the environment to artificially increased levels of naturally occurring radionuclides. For example, coal contains low levels of ^{238}U and ^{232}Th. Burning this coal for energy leads to both the release of some of these radionuclides to the environment and the concentration of the remaining nuclides in the resulting ash, leading to a material with an enhanced concentration of NORM with respect to the parent material, a technologically enhanced NORM (TENORM). Application of such residue materials, for example in construction materials, is actively encouraged as an environmentally friendly enterprise, however, the use of these TENORMs may have consequences in terms of radiation dose. In our current work we are developing and testing the relevance and applicability of a new LCA characterisation method, based on our recently published framework suggestion, to account for the enhanced exposure to NORM that can occur over the life cycle as a result of such processes. This has involved combining existing LCIA models with radiological models. We have extended, augmented and combined these models to develop characterisation factors for human health and ecosystems (freshwater, marine and terrestrial) for NORM radionuclides released to the environment and contained within construction materials. Our initial results suggest that for construction materials, NORM exposure may be the overriding direct impact to human health (when compared to human toxicity, particulate matter formation and ozone formation). As a corollary however, the radiation doses resulting from enhanced exposure to NORM in construction materials are lower than those resulting from many lifestyle factors and common medical procedures. In this poster we present the application of our novel impact assessment method and use it to highlight industries and product systems that are likely to be affected by the issue of NORM exposure.

TU224

How sustainable are renewable and green chemistry raw materials according to LCA ?

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Sourcing raw materials from vegetable origin and/or derived from green chemistry are concepts widely accepted as sustainable. In its recently published sustainability program, Sharing Beauty with All, the L'Oréal Group has selected many indicators to ensure that all its new cosmetic products will be environmentally improved. Among these indicators, the use of renewable raw materials sustainably sourced or derived from green chemistry in formulas are key elements of the strategy to reduce the environmental footprint of the company. In 2015, more than 50 % in volume of raw materials used by L'Oreal were renewable. This represents around 1,400 ingredients from nearly 300 plant species sourced in over 80 countries. On the other hand, green chemistry is a soft and clean chemistry, aiming at a minimal environmental impact, thanks to eco-friendly production processes, renewable starting raw materials, and production of non-ecotoxic raw materials. On the other hand, Life Cycle Analysis (LCA) is a powerful multicriteria tool to assess the different potential environmental impacts due to a product or service. LCA is more and more used, and could soon be identified as a reference approach for environmental assessment: for example, at the European level, the PEF project (Product Environmental Footprint), is designing guidelines to ecoconceive chemical-based consumer products and create an environmental labeling. It is therefore necessary that all the efforts and actions undertaken by companies putting these products on the market and raw materials producers can be measured appropriately in LCA. However, some current LCA methodologies are still being improved, such as the ones related to biodiversity or resources. Furthermore, we do not always have the whole dataset in databases necessary to assess in details our raw materials. With the case study of the raw materials used by the L'Oréal Group in its cosmetic formulas, the aim of this work was to study how eco-designed raw materials are assessed by current LCA, what are their main impacts, and how their profiles are compared to chemicals from synthetic or petrochemical origin.

TU225

Life cycle assessment on bio-based polymer 2,5 furandicarboxylic acid from sugar beet cultivation

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In recent decades, there has been a lot of research focusing on reducing fossil-fuel dependence and building a more sustainable future through the use of polymers from renewable resources. Polymers have become an essential part of our lives since their properties are used to make a wide range of products in industrial applications such as packaging materials and the automotive industry. However, our increasing dependence on fossil-based polymer use has meant that the advantages they can bring have been overshadowed by their environmental disadvantages. Polyesters, containing both aliphatic and aromatic monomers, are one of the most promising family of polymers based on renewable resources and where an extensive number of papers have been published to date. However, limited industrial availability for some bio-based monomers, such as 2,5-furan-dicarboxylic acid (FDCA) in the production of polyester, continues to pose a challenge. The aim of this work is to develop the life cycle assessment of the bio-based polymer FDCA from sugar beet cultivation as a building block for plastics. The study was calculated using a "cradle to gate" assessment. System limits were created for the cultivation of the sugar beet up to and including the FDCA factory. This research of FDCA is divided into five sub-processes. The cultivation of sugar beet, the production of sugar beet to sucrose, the conversion of sucrose to fructose, the conversion of fructose into 5-hydroxymethylfurfural (HMF) and the oxidation of HMF into FDCA. This study has been developed by working with an industrial partner. Sugar beet, together with sugar cane, are the plants suitable for sugar extraction and they contain more sugar than any other plants. A previous study has shown that the carbon footprint range for beet sugar was found to be slightly lower than cane sugar imported and refined by around 606 and 700 $kgCO_2$/t sugar respectively. In the case of starch-based glucose and fructose syrups (among others), the available literature indicated a higher carbon footprint range than EU beet sugar by around 870 $kgCO_2$/t sugar. According to these results the key to success in this field of this investigation was using the sugar beet as a biomass resource. The detailed results from this study are still in a work in progress. However, the initial results provide significant reductions in the carbon footprint field during the different stages compared to other comparable aromatic fossil-based diacids like phthalic acid in terms of GHG emissions.

TU226

Carbon footprint of municipal solid waste collection

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Carbon Footprint (CF) is an environmental indicator used in life cycle assessment that allows measuring the total amount of CO2 emissions caused directly or indirectly by an activity or accumulated through the life cycle stages of a product (ISO 14064-14067). In the presented case study CF was used to analyze and assess the environmental impacts of the resources used for the collection of municipal solid waste by the company Contarina SpA. Contarina oversees waste management for the province of Treviso (Italy), serving about 260,000 appliances in 50 municipalities distributed in the territory. Thanks to its efficient door-to-door collection system, Contarina manages to recycle 72% of the collected waste. The presented case study assessed CF of year 2015 related the whole fleet involved in door-to-door collection of municipal solid waste. In addition, a future scenario, in which part of the current fleet is replaced by compressed natural gas engine (CNG) based vehicles, was assessed and compared to the current status. The environmental impact assessment was performed by adapting the SimaPro software from PRè and then integrated with economic figures considering costs of purchase, operation and management of the means used. The analysis aimed at improving sustainability of Contarina's services and its image in the frame of environmental sustainability while fostering an informed development and testing of new technologies aimed at reducing the overall greenhouse gas emissions generated by its waste collection services in the territory.

TU227

Estimating environmental impacts of mixed dishes: A case study on pizza

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Modern diets are largely comprised of mixed dishes, a mixture of components with varying proportions, which are not well studied in life cycle assessment (LCA). Here we explore the level of detail required to accurately determine environmental impacts associated with mixed dishes by deconstructing them into "basic components" for which life cycle invetory (LCI) is more likely to be available. We demonstrate two deconstruction methods using a case study on pizza.\nWe determined pizza consumption in the U.S. diet by identifying food described as "pizza" in the What We Eat in America 2009-2012 dataset. We deconstruct pizza consumption into its "basic components" by food pattern categories (FP, 37 components) and food ingredients (FI, 3,200 components). We use LCI datasets from the Ecoinvent v3.2 and World Food LCA Database v3.1 to match with FPs (datasets average) and FIs (direct match when possible).We use the ReCiPe Midpoint (H) V1.12 method to perform a climate change impact assessment. \nOn average, the daily U.S. diet contains 31 g/person-day that make up for 4% of the total energy intake (77 pizza items). The FP approach deconstructed pizza into 18 components resulting in a 5% intake overestimation mainly composed by grains (37%), cheese (27%), and vegetables (19%). This corresponded to a carbon footprint of 0.11 kg CO2 eq/person-day (3.5 kg CO2 eq/kg pizza), with the highest contributions from cheese (43%), meat (21%), and solid fats (21%). The FI approach identified 64 basic components associated with pizza and covered 98% of pizza intake primarily consisted of vegetables (27%), grains (25%), and cheese (18%). This resulted in a carbon footprint of 0.10 kg CO2 eq/person-day (3.3 kg CO2 eq/kg pizza) largely due to meat (54%) and cheese (25%). In this approach, 44 basic components were matched to LCI datasets, making up for 95% of intake. Our analysis did not consider impacts due to transportation, storage or cooking.\nWe demonstrate two methods to perform environmental impact assessment of mixed dishes with a case study on pizza. Preliminary results suggest that although these methods produce similar results, deconstruction and component contribution to impacts differs between methods; hence, the simplified FP approach should be interpreted with caution, especially for meat or dairy items for which impact per kg could vary substantially and require the higher FI resolution. In the future, we will consider more environmental impact categories in our analysis.

TU228

Influence of vermicomposts of agroindustrial origin in the production of biomass and quality of essential oil of Ocimum basillicum L.

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The disposal of solid waste is a serious environmental problem for humanity. Vermicomposting is used as one of the methods for recycling of organic waste, resulting in a humified material of great agronomic potential which promotes carbon sequestration when applied to the soil. In the cultivation of medicinal plants, the use of organic fertilizer is recommended, since it improves the chemical, physical and biological properties of the soil, and also correcting the possible deficiencies of macro and micronutrients in the soil. The aim of this study was to evaluate the influence of a vermicomposted cattle manure (CM), orange peel (OP), and filter cake (FC) in the cultivation of basil *Ocimum basilicum* L. in two tropical soils, from Brazil: a Latosol, typical soil in the Brazilian Savannah (Cerrado); and a Vertisol, typical soil in the Pantanal floodplains. On the basil, the evaluated attributes were: the biomass production and the quality and the quantity of the essential oil extracted from the leaves. Three doses of vermicompost were applied: 15.0, 30.0 e 40.0 t ha^{-1}. After 60 days of cultivation, the aerial part of the plants was collected and the produced biomass quantified in the dried leaves. The essential oils from the leaves were extracted by hydrodistillation and the analyzes of their chemical composition were determinated through GC-MS. Vermicomposted CM mixed with OP or FC were, according to the data arrangements and statistical analysis, the best sample and treatment. **Latosol:** about the biomass production, it was not possible to observe a linear trend between the dosage of vermicompost and this attribute. The development of the basil plants which received vermicomposts were similar to the plants which received a mineral fertilization (commercial product) and significantly higher than the plants of the treatment of reference, basil cultivated without adubation. **Vertisol:** the effect of the vermicompost in the biomass production was masked, probably due to the higher content of natural organic matter naturally in this soil. The GC-MS analysis of the essential oil extracted showed that the organic fertilization influenced positively the production of the main component of the essential oil, the linalol, that increased its content according to the increase of the applied dosage of vermicompost, in the two studied soils. The highest linalool content (34.90 %) was quantified in the basil cultivated with vermicomposted FC + CM (30.0 t ha^{-1}), in Vertisol.

Assessment of PBT and vPvB chemicals: Requirements, challenges and policy implications (P)

TU229
A benchmark-level approach for evaluating PBT and vPvB chemicals in REACH authorisation and restriction procedures
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A key objective of the European chemicals legislation REACH is to ensure that the risks caused by substances of very high concern (SVHC) are adequately controlled. The two regulatory procedures adopted in REACH to control the risks arising from SVHCs are authorisation and restriction. While the aims, triggers and decision steps (including responsibilities of actors involved) differ between these regulatory instruments, they both make use of socio-economic analyses (SEA), which is generally defined to be a tool(box) for assessing all relevant positive and negative impacts from substances' use or non-use, and for comparing these impacts across different scenarios. It is crucial to acknowledge that the regulatory concern of persistent, bioaccumulating and toxic (PBT) as well as very persistent and very bioaccumulating (vPvB) substances differs categorically from those of other SVHCs. In particular, for PBT/vPvB substances the impacts result from long-lasting exposure. We suggest a benchmark level approach for the evaluation of PBTs/vPvBs which links the reduction of impacts from a certain PBT/vPvB abatement measure (or a combination of measures) with the corresponding costs of abatement. The assessment of impacts is based on dynamic modelling of expected PBT/vPvB concentrations in different environmental media and over time. This is crucial in order to account for the environmental distribution patterns of PBT/vPvB substances over time, and thus for the time course of impacts. We discuss how PBT/vPvB exposure dynamics can be transformed into time-related impacts for different environmental media and for humans via the environment. The costs of PBT/vPvB abatement are determined by means of a cost-effectiveness analysis approach. For every control measure (e.g. a ban, a clean-up measure, a remediation action, etc.) an estimate of costs per unit of emission/exposure reduction (expressed in, for example, €/kg emissions reduced), denoted 'cost-effectiveness ratio', is determined. This allows constructing a marginal abatement cost curve for (a group of) PBT/vPvB chemicals. We discuss how impact estimates can be transformed into benchmark levels of PBT/vPvB avoidance. These benchmark levels can be compared with marginal costs in order to conclude on the proportionality of (combinations of) abatement measures.

TU230
The Bioaccumulation Assessment Tool: An Organizational Framework for Bioaccumulation Assessment
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Thousands of chemicals are under evaluation for potential hazard, exposure and risk to ecological receptors and humans. Screening methods include the application of Persistence, Bioaccumulation and Toxicity (PBT) criteria against available data. A variety of regulatory programs require Bioaccumulation assessment, e.g. Europe's REACH legislation, the Canadian Environmental Protection Act (CEPA), and Japan's Chemical Substances Control Law. The multitude of metrics for assessing bioaccumulation include: the octanol-water partition coefficient (K_{OW}); bioconcentration factor (BCF), biomagnification factor (BMF) and the total elimination half-life (HL_T) typically determined under controlled laboratory conditions (e.g., OECD 305); and the BMF, bioaccumulation factor (BAF), and the Trophic Magnification Factor (TMF) measured in the environment. In addition to laboratory and field measurements, these metrics can be calculated by quantitative structure-activity relationships (QSARs) and bioaccumulation models. There are various quantitative criteria and quantitative and qualitative thresholds for the previously listed bioaccumulation metrics, e.g., REACH Annex XIII; however, there are no well-defined implementation strategies that include all of these lines of evidence. The objective of this project is to develop a user-friendly, organizational framework and computational tool in the form of an Excel/VBA spreadsheet to integrate various lines of evidence using a quantitative weight of evidence (QWOE) approach to guide bioaccumulation assessment. The Bioaccumulation Assessment Tool (BAT) brings together measured and modelled data, e.g., chemical properties, *in vivo* data (BCFs, BMFs, HL_T, absorption efficiency), *in vitro* data, and *in silico* data (BCF-QSARs, biotransformation rate constant QSARs). These multiple lines of evidence are treated as "Input". Primary "Output" from the BAT includes a suite of bioaccumulation assessment endpoints presented against user-defined criteria/thresholds and data for benchmark chemicals enabling the assignment of bioaccumulation classifications (e.g., "B" or "not B"). The BAT seeks to facilitate transparent and consistent bioaccumulation assessment and provide strategic guidance for integrated (tiered) testing to address uncertainty. The development of the BAT includes multi-stakeholder participation and the BAT will be freely available.

TU231
BCFmax as a new screening indicator for the bioaccumulation potential of organic chemicals - The case of linear alkylbenzenesulfonate (LAS) surfactants
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The PBT/vPvB assessment of organic chemicals requires quantitative information on their bioaccumulation potential. A high bioconcentration potential in terms of BCF values > 2000 corresponds to a hazard-based concern that may result in the restricted use of these compounds. However, experimental data for this endpoint are costly and therefore often not available for a majority of chemicals! Here we introduce the screening indicator BCF_{max} as a new trigger for experimental data, applied to ingredients of every day products, such as the most widely used anionic surfactants in household cleaning products, namely LAS. For these chemicals, high consumptions > 1000 tons/a are in contrast to the broad absence of experimental BCF values. However, acute toxicity data for the base set of the standard toxicity tests (i.e. Daphnia, Algae and fish) are usually available. Using the established concept of lethal body burden and assuming a universal lethal membrane concentration of 100 mmol/kg, a maximum BCF is estimated from exiting toxicity data. For compounds with a non-specific mode of action (MOA), the predicted BCF corresponds well with the experimental BCF, while the predicted BCF is overestimated for compounds with a specific MOA. It follows that in case of a maximum predicted BCFs below 2000, no experimental study is deemed necessary! In case of four anionic surfactants (i.e. C10-C13 LAS) with known BCFs, the predictions are within a factor of five, indicating that the observed toxicity of this group of compounds is mainly attributed to their bioaccumulation potential.

TU232
PBT assessment of substances - Proposal of a trigger value for bioaccumulation in terrestrial oligochaetes
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Assessment and regulation of PBT (Persistent, Bioaccumulative and Toxic) substances, are necessary to ensure a high level of protection of human and animal health, and of the environment. In aquatic organisms, trigger values for the identification of bioaccumulative ("B") and very bioaccumulative ("vB")

substances are bioconcentration factors (BCF) of >2000 and >5000, respectively, obtained from fish flow-through studies according to OECD 305. However, Annex XIII of the REACH regulation does not define similar trigger values for bioaccumulation in terrestrial organisms. Considering the availability of a standardized test guideline on bioaccumulation in terrestrial oligochaetes (OECD 317), the question arises whether and how the BAF and BSAF values from such earthworm bioaccumulation tests can be related to BCF criteria from fish bioconcentration tests. In this context, the objective of the present work is the determination of a trigger for bioaccumulation in terrestrial organisms. For this aim, the study comprised the following three steps: Literature research on available bioaccumulation factors (BAFs) both in open scientific literature and in regulatory data from several OECD 317 studies and performance of correlation analysis between soil-/substance-properties, BCF and BAF values. Performance of bioaccumulation studies according to OECD 317 with the earthworm *Eisenia andrei* using the four model substances endosulfan, methoxychlor, o-terphenyl and PCB153. Proposal of a trigger value for identification of bioaccumulative substances in terrestrial organisms based on the literature research and the performed experimental results. The results clearly showed that organic carbon content of the test matrix used in the tests influences the BAF, whereas no clear correlations between log K_{OW}, log K_{OC} or similar substance properties and the BAF were observed. Additionally, no correlation was observed between substance-specific BCF from fish studies and BAF determined with earthworms. Therefore, lipid- and C_{org}-normalized BSAF should be used for the assessment of terrestrial BAF. Kinetic BSAF from both experimental studies and literature-derived values ranged from 0.21 to 14.8. Based on the data evaluated in the present work, a BSAF trigger value of 1.00 is proposed as a general trigger to indicate bioaccumulation in terrestrial organisms. Other aspects like non-depurated residues at the end of the elimination phase are discussed.

TU233

Impact of revision of the ECHA guidance on PBT assessment of chemicals

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The revision of the ECHA guidance on PBT assessment of chemicals (Chapters R.11, R7b, R7c) includes several factors that make the assessment much more conservative and which will drastically increase the number of chemicals for which one or more PBT criteria are triggered. The main impact concerning the P-assessment will be caused by the confirmation of the reference temperature of 12 °C at which simulation studies should be performed or to which half-lives determined at different temperatures should be normalised. In comparison to a reference temperature of 20 °C, which was used for criteria setting and which is recommended in the simulation test guidelines, the trigger half-lives are reduced by a factor of two. For the P-assessment in water the pelagic OECD 309 test with minimum suspended solids is listed as the preferred option whenever technically feasible. This would characterise many substances as being persistent even if water is not a relevant compartment for degradation due to rapid partitioning into the sediment. According to the revised guidance, enhanced simulation tests that include environmentally relevant processes (e.g. photolysis), and that have the potential to show non-persistency under realistic conditions, should not be used. A very conservative worst-case approach is followed for NER, which in a first instance should be regarded as non-degraded parent substance. For the B assessment the BCF is considered the most important parameter. According to ECHA guidance, a BCF study should be conducted whenever technically feasible even in cases where bioaccumulation via water is not the most relevant uptake route. This approach is seen as highly problematic because it could lead to cases where more realistic and scientifically justified experiments (e.g. biomagnification studies) as well as other higher tier approaches like modelling are not used to an extend they should be used. No clear guidance is given for a Weight-of-Evidence approach in the revised guidance. Although it should be used to additionally characterise substances as PBT it seems not to be possible to supersede PBT classification based on very simple lower tier studies by higher tier assessments showing that the substance is not PBT. Based on the revised ECHA guidance many substances that do not cause non-acceptable unpredictable long-term effects will be classified as PBT substances.

TU234

Quality of Information Requirements in REACH Registration Dossiers of High Tonnage Chemicals and Implications on the Identification of PBT/vPvB-Substances

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'No Data no Market' is the slogan of the European Union's regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [1]. Data are also required for the assesment of PBT or vPvB properties of chemicals. Chemicals produced or imported to the European Union's market above one tonne per year have to be registered with the European Chemicals Agency (ECHA). Therefore, information requirements depending on produced or imported volume per year are submitted to ECHA. Availability of data is reviewed either IT based within the submission by a technical completeness check or with regards to form and content in compliance checks by ECHA. But compliance checks are limited to 5% of the registration dossiers. With the aim to evaluate systematically the data availabilty and quality for high tonnage chemicals in registration dossiers both a screening [2] and a formal check approach were developed and applied. In the first screening step, the availability of standard information according to REACH Regulation (column 1 of ANNEX VII to X) was evaluated with decision trees in a web-system for managing data on substances [2]. It is also permitted to waive or adapt the standard information by specific rules (REACH, column 2 of ANNEX VII to X or Annex XI [1]). These waivings or adapations were not concluded with the first screening approach. Subsequently, a formal check addressing formal conformity with the specific rules justifying data waiving or adaptations, *e.g.* read across/grouping approaches and (Q)SAR models, was conducted. [1] European Parliament, European Council. 2006. Regulation (EC) No 1907/2006. Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). OJ L 396, 30.12.2006, p. 1. [2] Springer A, Hermann H, Sittner D, Herbst U, Schulte A. 2015. REACH Compliance: Data availability of REACH registrations. Part 1: Screening of chemicals > 1000 tpa. Federal Institute for Risk Assessment, Berlin, Germany, and Federal Environment Agency Dessau, Germany. Texte / Umweltbundesamt 15/43.

TU235

The UK Verification Laboratory Testing Programme of chemical hazard assessment for offshore chemicals - A summary of the first three years

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In the UK, the Department for Business, Energy and Industrial Strategy (BEIS) is tasked to ensure that chemicals used by the oil and gas exploration and production industry on the UK continental shelf (UKCS) do not pose an unacceptable risk to the marine environment. All chemical products used offshore must be registered in accordance with the OSPAR Harmonised Mandatory Control System (HMCS), using high quality persistence, biodegradability, and toxicity (PBT) data for the individual substances used in each formulation. The regular verification by Cefas of test results from selected products was implemented to ensure that this high quality of registration data was supported by robust evidence. Over the last three years, 85 tests on 11 substances have been carried out and the results compared with data submitted for registration. In this poster, an analysis of the results is presented, indicating the level of agreement that has been found between the verification studies and the registration data, and the main implications are summarised.

TU236

Implementing the hydrocarbon block method for PBT assessment of petroleum substances

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One of the European Union's REACH Regulation requirements is to perform a Persistence, Bioaccumulation and Toxicity (PBT/vPvB) assessment for substances brought on the market in the EU. This represents a challenge for complex substances (e.g. UVCBs). For petroleum substances, the PBT/vPvB assessment is performed using the hydrocarbon block (HCB) method, which uses information from representative constituents/structures grouped by hydrocarbon class and carbon number. A systematic review of the persistence and bioaccumulation properties of the main HCBs was conducted, and those HCBs deemed potentially to meet the persistence and bioaccumulation criteria were subsequently assessed for toxicity. The strategy to assess PBT/vPvB properties of HCBs consisted of the following steps: QSAR models were applied to compute predictions of primary biodegradation half-lives in water (P) and fish bioconcentration factors (B) for a Concawe library of representative structures, as recommended in ECHA REACH Guidance Chapter R.11. Two modules from EPISuite v4.11 were used to screen for persistence and bioaccumulative properties: BioHCwin and BCFBAF. Where available for hydrocarbons, experimental primary biodegradation half-lives in un-acclimated freshwater or marine water, as well as aqueous bioconcentration and dietary bioaccumulation test data were compiled and compared to model predictions for the constituents. An assessment of the potential persistence in soil and sediment was performed by estimating soil and sediment half-lives from aquatic half-lives using a 1:1:4 water:soil:sediment conversion factor. Measured data and model predictions for P and B properties were evaluated for the representative constituents of an HCB to develop an evidence-based conclusion regarding P and B. If an HCB was found to contain representative structures that potentially met the P and B criteria, toxicity was evaluated in accordance with

Annex XIII criteria. Aquatic toxicity was assessed on the basis of available measured data and predicted data using the target lipid model. Mammalian criteria were assessed by consideration of the presence of Carcinogenic, Mutagenic, Reprotoxic and/or STOT RE classified substances within a block that would be identified as potentially 'T'. This poster will present the results of the PBT assessment for HCBs.

TU237

PBT Assessment of Pharmaceuticals - Evaluation and Challenges

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Persistent, bioaccumulative and toxic (PBT) substances or very persistent and very bioaccumulative (vPvB) substances are compounds with hazardous properties. The non-biodegradability (persistence) and high accumulation in organisms (bioaccumulation) may elicit long-term adverse effects in the environment. Persistent and bioaccumulative substances concentrate in the environmental compartments (water, sediment, soil, air) and can be distributed in the food chains. Ecotoxicological effects are strengthened by bioaccumulation and often appear in remote areas like marine and polar regions. In the framework of PBT assessment, contrary to the risk assessment, the identification of PBT substances has to be performed regardless of the emission into the environment. The poster presents an evaluation of medicinal active ingredients (human and veterinary pharmaceuticals) under assessment at the German Environment Agency (UBA). Less than 10 % have been identified as potential PBT candidates. Due to data lacks in many cases a definite PBT classification is not possible. The PBT candidates amongst veterinary phamaceuticals are mainly antiparasitics, whereas human pharmaceuticals cover a wider spectrum of different pharmaceutical groups. Whereas the PBT assessment for veterinary medicinal products is included in the benefit-risk assessment, PBT properties are not considered in benefit-risk assessment for human medicinal substances. This means, identified PBT properties have no consequences for the authorisation of human pharmaceutical products. Open issues in current regulations are addressed, e.g. there are no approaches for ionisable substances, consideration of additional endpoints for bioaccumulation (BMF, BAF, BSAF, in-vitro tests, field data), no trigger values for bioaccumulation and ecotoxicity studies with terrestrial organisms available.

TU238

Streamlining for relevant information on metabolites with regard to PBT criteria

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Under Regulation (EC) No. 1907/2006 (REACh) a conclusion can be drawn that a substance fulfils the Annex XIII criteria (PBT, vPvB criteria) if degradation products or metabolites with PBT or vPvB properties are identified (ECHA Guidance R.11). The assessment of metabolites with regard to the Annex XIII criteria can be a challenging process, as firstly, often appropriate identification of metabolites by tests on simulation of biodegradation is missing, and secondly, the subsequent screening for information on the PBT behavior is complicated in itself by facing the use of a large amount of databases and tools. With regard to this objective we present a way forward on how to tackle these challenges. Several steps are described to streamline relevant information. Starting with the identification of a metabolic pattern, followed by identification of relevant metabolites and subsequent in-depth screening on PBT properties; this exercise comprises the use of publicly available databases and *in silico* tools such as the OECD toolbox, EAWAG BBD/PPS, VegaNIC and other tools. It is indicated that no single optimal way exists but that by combination of certain key information from multiple models and databases a comprehensive and reliable conclusion may be drawn.

TU239

Assessment of persistence and mobility of REACH-registered chemicals - The influence of input parameters, evaluation criteria and concepts

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Anthropogenic chemicals that are environmentally stable and highly polar (mobile in water) have the potential to break through natural and technical barriers in the aquatic environment and thus threaten the quality of our raw water resources [1]. Such persistent (P) and mobile (M) organic chemicals (PMOCs) are especially problematic if they also possess a high potential to be released into the environment and are toxic to humans or ecosystems. It is thus of vital scientific and regulatory interest to predict and assess P and M of chemicals in commerce. A wealth of property data on chemicals exist in the open or grey literature, either experimentally derived or based on QSAR-modeling. Furthermore, in Europe chemicals have to be registered by the manufacturers under the REACH legislation. In this process chemical property data have to be provided to the European Chemicals Agency (ECHA), besides other information. We have compared the evaluation of P and M for 150 REACH-registered chemicals via three different approaches: 1) The approach by Kalberlah et al. [2] based on a decision tree in which a number of questions on chemical properties have to be answered to come to a conclusion; 2) The approach taken within the European research project PROMOTE in which publically available property data were used and gaps in experimental data were filled with QSAR evaluations. 3) Our own expert judgment based on both open data and data from the REACH dossiers. Considerable inconsistencies in the P and M assessment between the three concepts were revealed. The reasons for these inconsistencies are manifold, including differences in the definition of P and M and uncertainties both in experimental data as well as in QSAR models. The poster will discuss this difficulty and its impact on the potential to regulate chemicals; as well as ways forward. [1] Reemtsma T., Berger U., Arp H.P.H, Gallard H., Knepper T.P., Neumann M., Quintana J.B., de Voogt P. (2016) *Mind the gap: Persistent and mobile organic compounds – water contaminants that slip through*, Feature Article, Environ. Sci. Technol. *50*, 10308-10315. [2] Kalberlah, F., Oltmanns, J., Schwarz, M., Baumeister, J., Striffler, A. (2014) Guidance for the precautionary protection of raw water destined for drinking water extraction from contaminants regulated under REACH. Project (UFOPLAN) FKZ 371265416, Umweltbundesamt, Dessau.

TU240

Screening for emerging persistent and mobile organic water contaminants by liquid chromatography and supercritical fluid chromatography coupled to mass spectrometry

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The release of persistent and mobile organic chemicals (PMOCs) into the aquatic environment is a risk to the quality of water resources. As a consequence of their high polarity, PMOCs can pass through wastewater treatment plants or drinking water purification processes and cross natural barriers [1]. Until now only a few such compounds are known, mainly due to analytical challenges in their determination, but we assume that their number is much larger. The aim of this work was to screen for hitherto unrecognized PMOCs in drinking water relevant raw water samples, using analytical methods suited for highly polar chemicals. The target compounds were a set of 70 REACH-registered chemicals that have previously been shortlisted as potential PMOCs within the European research project PROMOTE. A screening procedure based on solid-phase extraction (SPE) and liquid chromatography – triple quadrupole mass spectrometry (LC-MS/MS) or, alternatively, supercritical fluid chromatography – high resolution mass spectrometry (SFC-HRMS) was established. A total of 20 European water samples (groundwater, surface water, reversed osmosis permeate and concentrate and sewage treatment plant effluent) were extracted on 4 SPE-materials (Oasis MCX, Oasis WAX, ISOLUTE® ENV+, Supelclean^{TM} ENVI-Carb) before separation on either a polar modified reversed-phase column or a porous graphitic carbon column (for LC), and an Ethylene-Bridged Hybrid (BEH) or a Torus Diol column (for SFC), respectively. Of the target PMOCs, approx. 30 were detected in at least one sample, among them novel PMOCs, but also a number of known water contaminants such as melamine, tris (1-chloro-2-propyl) phosphate, saccharin, dapsone, bisphenol S, and acesulfame. The present study has qualitatively identified a considerable number of emerging PMOCs in drinking water resources. For the more frequently detected novel PMOCs a more detailed investigation of their sources and behavior will be required to assess their possible risk for drinking water quality. [1] Reemtsma, T., et al., Mind the Gap: Persistent and Mobile Organic Compounds - Water Contaminants That Slip Through, *Environ. Sci. Technol.*, 2016, 50: 10308-10315

TU241

Using REACH registration data for the identification of persistent, mobile and toxic (PMT) substances

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Chemical substances and their uses that fall within the scope of the REACH Regulation (1907/2006 EG) have to be registered at ECHA in Helsinki. Registrants are requested to ensure a high level of protection of human health and the environment. By doing so, industry guarantees the safe use of chemicals throughout the whole life cycle. Raw water resources for the production of drinking water need a high level of protection. In Europe drinking water is obtained mainly from groundwater, reservoirs or river bank filtration. If these environmental compartments are exposed to hazardous chemicals a contamination of drinking water is possible. In the last decade the fate and behaviour of polar substances has been investigated both scientifically and from a regulatory perspective. The finding is that their intrinsic hazard potential is maximised if they are at the same time persistent in the environment and mobile in the water cycle. Once emitted, these substances remain in the aquatic environment and the contamination is irrepairable. If in addition they also fulfil the properties of being toxic emissions into water resources should be avoided during the production and downstream use of the substances. A proposal for an assessment concept of persistent, mobile and toxic chemicals (PMT substances) has been presented by Kalberlah et. al (2015). We applied the PMT assessment concept to a range of chemicals that are registered at ECHA. Here we present the result and name substances with PMT-properties. We show that the assessment concept for PMT substances uses the same set of data provided as standard information requirements for the registrations under REACH. The proposed criteria and the assessment concept may builds the basis for regulatory measures by authorities. From an authority point of view the intrinsic substance properties persistency, mobility in the water cycle and toxicity (PMT) may be of equivalent concern as those substances with persistent, bioaccumulative and toxic (PBT) properties. *Fritz Kalberlah et al (2015): Guidance for the precautionary protection of raw water destined for drinking water extraction from contaminants regulated under reach. Project no. 371265416 of The Federal Ministry for the Environment (BMUB) in Germany*

TU242
An overall picture of Risk, Exposure and Hazards in a regulatory context
X. Trier, European Environment Agency
For the past decades the regulation of single substances, by horizontal and thematic EU regulations has provided some significant improvements in the reduction of environmental pollution, and has provided improved qualities of e.g. bathing water and air. However, environmental and health policies generally remain to address the total sum of chemical pressures from the total exposure to mixtures of chemicals, which is what affect the health of ecosystems and humans. Given that it is unlikely that all known and unknown chemicals ever will be fully tested for their combined hazards and exposure pathways in a bottom-up approach, this presentation will ask which other options there is to manage the risk of chemicals? It is proposed, that the most risky *known* substances at least could be removed, such as: 1) substances qualifying for being Substances of very high concern (SVHCs); 2) substances contributing most to exposure, and 3) planetary boundary threat substances. Persistent chemicals is one group of such high risk substances contributing to exposure: If bioaccumulative they will accumulate internally in living organisms, but if mobile and persistent (PM) they can be difficult to remove from e.g. in effluents from WWTP or from air. Instead they and the pseudo persistent substances will accumulate in the external environment such as water, which has been seen in the substitution from long to short chain fluorinated substances. Changes in use patterns can also aggravate the accumulation and/or the exposures, e.g. when water, sludge and articles are recycled more, due to scarcity of resources. Finally the presentation will identify gaps in knowledge and in indicators that could be used to assess the overall risk – leading to the question of asks which overall harm from chemical pressures on humans and the environment that we as a society is willing to accept?

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective (P)

TU243
Health risks assessment from the exposure to trace metals in urban environments
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Urban activities release potentially harmful pollutants to the environment, especially metals and organic compounds. Thus, we are continuously exposed to these pollutants in urban environments. Road dust and soil from three urban areas with different population densities were collected and size fractions were analysed for metal content (Co,Cu,Cr,Ni,Pb and Zn). The main objective was to elucidate which material, soil or road dust, poses higher health risk for children or adults. Results showed similar distribution of the size particles among cities, predominating fractions between 75µm and 2000µm in road dust and below 75µm in soil. Metals were mainly bound to Particulate Matter with 10 micrometers or less in diameter (PM10) in both soil and road dust. This kind of particles has an inhalable sizeincreasing the potential risk of adverse health effects through the incorporation of metals to the respiratory tract. The risk assessment showed that the most hazardous exposure pathway was the ingestion via, followed by dermal absorption and inhalation routes. Values of hazard quotient showed that the risk for children due to the ingestion and dermal absorption was higher than for adults, and slightly larger at PM10 comparing to < 75µm fraction for the inhalation route. Although higher risk was found for road dust,neither the hazard index nor the cancer risk index value overreached the safe value of 10-6.

TU244
Health Risk Assessment of Heavy Metal Exposure to Street Dust in Mexico City
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In order to quantify the toxicity of metal bounded to human health, through the assessment of incremental life time cancer risk (ILCR), due to the presence of dust in the streets of Mexico City, more than one hundred of samples were collected along 12 km of a main street. This street crosses the whole city from North to South, with different traffic intensities, where more than 40 elementary schools are located no more than 200 meters away from the street. The metal extraction of dusts was carried out by acid digestion followed by atomic absorption spectroscopy analysis and scanning microscopy analysis. Principal component analysis showed that the main sources are crustal and vehicle traffic followed by industries. The abundance of metals in all sampling sites was Fe> Pb> Cu> Zn> Cr >Mn > Mo >Cd. Concentration of metals ranged between 64027 to 36734, 239 to 83, 128 to 67, 95 to 49, 76 to 37, 64 to 28, 44 to 18 and 2 to 5 mg/kg respectively. Cancer risk assessment was carried out by using the information provided by the Integrated Risk Information System (IRIS) of the USEPA. Monte Carlo simulation was performed to investigate the uncertainty and variability involved, and the impact on quantification of expected ILCR. The ingestion is the main exposure route for heavy metals followed by inhaled resuspended particles, whereas dermal absorption seems to be almost negligible. The cancer risk by exposure to Pb and Cr were larger than the USEPA guideline line, and children have a potential risk due to the hazard index of Pb and Cd greater than the safe level. Pb presented the highest toxicity in most of the sites. Sensitivity analysis showed that exposure duration has the highest contribution to the variance in risk estimation of toxic metals.

TU245
Consumers can lower their toxic arsenic intake by selecting appropriate kitchen practices
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In contrast with the high number of studies reporting Arsenic (As) speciation data in various unprocessed food items, food processing studies are limited to few matrices and speciation is not always given attention. The goal of the present study was to gain insights in the effects of food preparation on As concentrations and speciation of different kind of foods. In this way, a more accurate insight can be obtained in the actual effect of food processing on the exposure to As (species). In this study the concentration of total As and arsenic species (inorganic As, arsenobetaine, dimethylarsinate and methylarsonate) was monitored in different foodstuff (rice, vegetables, algae, fish, crustacean, molluscs) before and after processing using common kitchen practices. Potential leaching effects were separated from apparent effects due to changes in moisture content upon food preparation, by measuring water content of the foodstuff and by reporting arsenic concentrations on a dry weight base. Arsenic (species) did leach towards the broth during boiling, steaming, frying or soaking of the food. Concentrations declined with a maximum of 57% for total Arsenic, 65% for inorganic As and 32% for arsenobetaine. Based on a combination of literature data and our own results we conclude that the extent of this leaching tends to be species specific with inorganic arsenic leaching most easily, followed by the smaller organic molecules and the larger organic molecules.

TU246
Incorporating Selenium in Mercury related Fish Advisories
N. Gandhi, University of Toronto / Dept of Earth Sciences; G. Arhonditsis, S. Bhavsar, University of Toronto
Human exposure to mercury (Hg) from eating fish has been a major concern worldwide. Fish consumption advisories have been issued to protect human health. These advisories are typically “risk” based and neglect the benefit aspects of eating fish. As such, a reduction in fish consumption due to exaggerated risk concern may actually result in a health risk due to potentially low quality of replacement food items. Although it has been shown for half a century that selenium (Se) in fish can alleviate Hg toxicity when present in excess of Hg, such a protective effect is yet to be considered in fish consumption advisories. Using an extensive dataset of concurrent Hg and Se measurements in fish, we show that that despite wide geographic and fish species-specific variations in Se:Hg ratios, Se can be incorporated into Hg advisories using a simple approach without requiring much additional data, and substantially more fish can be safely consumed than currently advised. Our suggested approach, which can also simplify advisories and lead to a greater reception by consumers, is likely applicable to both freshwater and marine fish worldwide.

TU247
Mercury (Hg) concentrations in predatory bird livers and eggs as an indicator of changing environmental concentrations
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Concern over the health and environmental effects of mercury (Hg) has prompted

an international agreement, the Minamata Convention on Mercury. This aims to control anthropogenic releases to the environment and reduce potential impacts on humans and wildlife. Monitoring is one strand of evidence needed to determine to what extent the convention is successful. The Predatory Bird Monitoring Scheme (PBMS - http://pbms.ceh.ac.uk/) has monitored long-term trends in environmental Hg concentration in sentinel predatory bird species in the United Kingdom with the aim of detecting changes in contaminant exposure at a national scale. We explore how such monitoring can provide an evidence base by which the impact of the Minamata Convention can be assessed for different habitats across Britain. Specifically, we demonstrate how monitoring Hg concentrations in sparrowhawk *(Accipiter nisus)* livers and failed golden eagle *(Aquila chrysaetos)* eggs provide a means of detecting change in bioavailable Hg in terrestrial lowland and upland habitats, respectively. We found liver Hg concentrations in sparrowhawks vary with factors such as age, sex and body condition and that long-term monitoring data must take such variation into account. With respect to Hg in eagle eggs, we also show that stable isotope concentrations can be used to distinguish between birds that accumulate Hg through terrestrial or marine trophic pathways. Finally, we examine the magnitude of change from levels of current Hg contamination that we are able to detect statistically using predatory birds as sentinels.

TU248

Hair as a matrix for assessing mercury concentrations in polar bears, Ursus maritimus: Variability along single hairs and location on the bear

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Mercury is biomagnified along aquatic food chains and we investigated the use of polar bear hair as a non-invasive matrix for the assessment of mercury concentrations in top predators. From each of 7 Greenland bear skins, 18 hair samples were collected; six samples were taken along each of three head-to-tail transects on the back and left and right side of the animal. Each hair sample – lengths between 4 and 16 cm - was cut into 1 cm fractions and the concentration of total mercury was determined by means of a Milestone Direct Mercury Analyzer (DMA-80). Mercury concentrations in the hair samples varied between 2 and 27 μg Hg g^{-1} - with most samples in the range 5 to 10 μg Hg g^{-1}. Bears shot in March and May generally had higher mercury concentrations than bears shot in July and September. For some bears, mercury concentrations in the dorsal hair decreased significantly along an axis from head to tail, but otherwise no clear differences in the mercury concentrations of the hair samples taken at different positions on the individual bears were demonstrated. The hair of some of the bears showed consistent trends of increasing or decreasing mercury concentrations going from the base to the point of the hair; the interpretation of these trends is hampered by the fact that we know too little about the onset of hair production and the growth rate of the hair in polar bears. The study includes a protocol recommendation for sample collection and laboratory work for measurements of mercury in polar bear hair. Based on literature values for the correlation between mercury concentrations in hair on the one hand side and liver, kidney, brain, muscles, blood and brain on the other, total body burdens for total mercury, methylmercury and inorganic mercury (Hg^{2+}) were estimated for the bears of the present investigation. From this, we estimated the percentage of the total mercury body burden polar bears may excrete in one molt of hair. Polar bears from this study could have body burdens of between 86 and 312 mg total mercury and in a full molt, they could deposit between 39 and 63% of their body burden in their hair. Polar bears could have body burdens of methyl mercury between 55 and 185 mg Hg. The polar bears could deposit 65 to 88% of their methyl mercury body burden in their hair in a full molt. Body burdens for Hg^{2+} were calculated to between 32 and 127 mg Hg^{2+}.

TU249

Trees and microbes at a Hg contaminated chlor alkali facility

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Large dumps that are used to store industrial tailings can generate significant unvegetated surfaces after they are abandoned, suggesting that these regions become biologically infertile. Revegetation of the tailings dumps produced by various industrial activities is thus necessary to prevent dust storms and erosion and represents a great challenge for ecological restoration. However the fate of Hg into the woody vegetation cover remains unclear. Also, little is known about the microbial colonisation and community structure of revegetated tailings following site exploitation. In this study, we investigated the historical evolution of Hg concentrations in tree rings from trees growing on and near a landfill that received dredged sediments from a Hg cell chlor-alkali facility in France. We were able to demonstrate the incorpoation of Hg into perennial tissues, that were located near the dump, and that may serve as reliable indicaotr of past Hg exposure. We furtherdemonstrated that most of the Hg detected in the aboveground parts of *Salicaceae* trees collected at that site had entered the poplar leaves through exclusively through an atmospheric pathway. We also characterized the microbial communities that had naturally recolonized the sediment of a chlor-alkali tailings dump after sediment deposition had ceased, and the microbial communities from a recently implemented field trial. These studies will provide an improved point of reference for microbial ecology and plant research in tailing environments, and our findings will be valuable in designing future ecological restoration programs.

TU250

Mercury accumulation in freshwater surface and bottom biofilms

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Microorganisms living in biofilms are well known to accumulate mercury (Hg) present in their ambient environment and to further transform it through (de)methylation and reduction processes. They also represent an important point of Hg entry in autotrophic-based food chains. The present study aimed thus to better understand Hg accumulation process in biofilms, notably the influence of biofilm composition. To that end, biofilms were colonized on artificial substrata in the Versoix River (Switzerland) for three months. Due to different light exposure (induced by different colonization depth), two distinct biofilms were obtained (so-called *surface* and *bottom* biofilms). Both biofilms were brought back in the laboratory and exposed to 93 ± 26 pM ^{199}Hg during 72 h using microscoms. Their Hg concentrations (total Hg and non-extractable Hg e.g. obtained after a washing step with cysteine) were measured at different step time (10 min, 30 min, 2 h, 6 h, 24 h and 72 h). Their compositions were also analysed with the determination of their chlorophyll a content and ash-free dry weight as well as with the characterization of their thiols (colloidal and capsular) contents in their exopolymeric substances (EPS) matrix. The exposure media were also analysed for their physico-chemical parameters, and dissolved inorganic and organic Hg concentrations. The surface biofilms were observed to have a higher biomass and chlorophyll a content than the bottom biofilms. Additionally, the capsular EPS thiol concentrations were found to be higher in the bottom biofilms as compare to the surface biofilms. The non-extractable Hg concentrations as a function of time were modeled in both biofilms with one compartment non-linear pseudo-first order kinetics. The calculated uptake and clearance rate constants of the bottom biofilms were found to be 10 and 2.3-fold higher, respectively, than those of the surface biofilms, possibly due to a thicker matrix in the surface biofilms. The capsular fraction was observed to decrease in bottom biofilms upon Hg exposure, which was mainly due to variation of the cysteine concentration. Hg was found to be mainly bound to the microorganism cell membranes and intracellularly accumulated with only 8% bound to EPS. That findings suggests that biofilms have an important role in the trophic transfer of Hg, especially in the benthic zone.

TU252

Assessment of the toxic effects exerted by chromium in soils combining standardized OECD tests and cell-based approaches in earthworms (Eisenia fetida)

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Chromium (Cr) is widely used in a variety of industrial processes which lead to releases into the different environmental compartments. Inputs of Cr into terrestrial environments could modify soil community and affect soil health. The standardized earthworm Acute Toxicity Test (ATT) (OECD-207) with *Eisenia fetida* provides information about which concentrations of Cr pose effects on growth and mortality in earthworms. Complementarily, parameters measured in earthworms immune cells (coelomocytes), could be an accurate tool to assess the toxic effects exerted by Cr in this soil dwelling organisms. OECD soils (OECD-207, 1984) were contaminated with 10 - 150 mg Cr/kg (Cr was added as $K_2Cr_2O_7$) and left stabilizing for 3 weeks. Healthy and clitellated *E. fetida* earthworms of similar size (400 ± 100 mg fresh weigh) were weight in tens and introduced in the test soils, maintained at 19 °C, in constant humidity and light conditions. For the ATT mortality and weight loss were measured (14 d) in order to obtain LC_{50} and EC_{50} values.. Cr was quantified in soils (EPA 3051A method), lixiviates (soluble fraction, DIN 38414-4) and earthworms (n=15) by AAS (SGIker General Service; UPV/EHU). Coelomocytes were extruded from exposed earthworms (3 pools of 5 individuals per treatment) and their number and cell viability were calculated. Cr concentrationsin lixiviates were very low and suggest the formation of Cr-soil complexes while Cr was accumulated in earthworms showing an increasing gradient from 40 mg Cr/kg onwards. Mortality and weight loss followed a dose response fashion. Exposure to the highest doses (130 - 150 mg Cr/kg) produced 100 % mortality. The LC50 value was calculated as 97 mg Cr/kg, which corresponded to 20 mg Cr/kg soluble fraction. The EC50 was 115 mg Cr/kg and corresponded to 22 mg Cr/kg (soluble fraction). Regression analyses showed a significant reduction of cell number with exposure concentrations ($R^2 = 0.97$, $p< 0.05$). Coelomocytes decrease could be a first sign of more severe toxicological effects at higher levels of biological complexity. The NR uptake capacity of coelomocytes appeared to be affected at concentrations higher than 40 mg Cr/kg. Effects reported in the present work could be related to responses at lower levels of biological complexity such as oxidative stress, DNA damage and apoptosis. *Acknowledgements* – Funded by Basque Government (Consolidated Research Groups, IT810-13) and BEC.AR (Program of scholarships for Argentine professionals).

Science communication and citizen science - strategies for successful stakeholder engagements (P)

TU253
The role of citizen science in monitoring small-scale pollution events
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Small scale pollution events involve the release of potentially harmful substances into the marine environment. These events can affect all levels of the ecosystem, with damage to both fauna and flora. Numerous reporting structures are currently available to document spills, however there is a lack of information on small-scale events due to their magnitude and patchy distribution. To this end, volunteers may provide a useful tool in filling this data gap, especially for inshore coastal environments with a high usage by members of the public. The potential for citizen scientists to record small-scale pollution events is explored using the UK as an example, with a focus on highlighting methods and issues associated with using this data source. An integrated monitoring system is proposed which combines citizen science and traditional reporting approaches.

TU254
Unbreak your science communication - public relations in environmental sciences are a matter of skilful marketing
T. Seiler, RWTH Aachen University / Ecosystem Analysis
Environmental science is more than other research fields connected to every day lives. Since we all live surrounded by the natural environment its health and quality readily impacts our well-being. As a consequence, findings from environmental research can raise major interest in the public, but are also prone to serious misunderstanding. Though our research can be deemed of high importance for society, attention for environmental sciences and coverage by the media is weak. Hence, we should start to actively communicate what we are doing and why this is important. But as our research is so closely associated with public opinions, fears and desires, many pitfalls lie ahead – and communication can go completely wrong if not done properly. This might be one strong reason for a widespread restraint in environmental sciences to engage in science communication. This presentation uses a rough categorisation shown by marketing expert Seth Godin in a 2006 Gel Conference talk, to identify the most prominent failures in science communication. The categories as introduced by Seth Godin describe failures in product design or customer loyalty, which he then terms “broken”. Looking at science communication as an approach to market research findings and consequent needs for politics and regulations, these categories also apply to communication activities. As in economy, broken relations to our target groups will reduce attention for our research findings, and hence our impact. Categories used to depict the difficulties of environmental science communication and suggest solutions are: 1. I am not a fish – regarding presentation design, 2. I didn’t know – regarding press releases, 3. Selfish jerks – regarding audience-specific communication, 4. Not my job – regarding press office communications, 5. Contradictions – regarding ambiguous statements, 6. The world changed – regarding new media, 7. Broken on purpose – regarding research articles. This list does not make a claim to be complete. The presentation rather points at general considerations behind proper science communication especially of environmental research. It aims to make the reader think and become aware of the major pitfalls. By clearly naming them and proposing solutions it should encourage environmental scientists to engage in stronger communication efforts in the future.

TU255
THE MOST DANGEROUS CHEMICALS IN PRODUCTS IN SERBIA: FIGHT TO KNOW!
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Modern chemicals management system was introduced into the Republic of Serbia in 2009 with the adoption of the Law on Chemicals, which is largely harmonised with the EU chemicals REACH Regulation. This regulation, *inter alia*, contain provisions on substances of very high concern (SVHC) and set down how consumers are entitled to be informed about the presence of SVHC in products if their concentration exceeds 0.1%. This means that producers, importers and distributers must share the information with the consumer that is needed to ensure the safe use of a product. By the end of 2015, not a single consumer in Serbia seems to have requested such information. The campaign the **“FIGHT TO KNOW!”** (in Serbian **“Izbori se da znaš!”**) was carried out in Serbia from October 2015 to April 2016 in order to test the implementation of the legal provisions providing consumer`s right to information on presence of SVHCs in products and to raise awareness about them. The subject of campaign included a total of **90 PVC products that can potentially contain SVHC phthalates**. Through communication with distributors, exercise of rights to information about the presence of SVHC in products was tested, while the actual content of phthalates in samples of selected products was determined through laboratory analysis. According to the results of the campaign, responses were received from 52.2% of the distributors, out of which 22.2% provided specific answers to the question about the presence of SVHC in products. **Only 10% of distributors showed high-level knowledge about SVHC and obligations related to legal provisions. Laboratory tests on contents of phthalates in selected products demonstrated the presence of phthalates from the List of substances of very high concern in 24 samples (26.7%).** The achieved results indicate low level of awareness about SVHC and related legal obligation to provide information on presence of these substances in products. This campaign was a part of the project **“Capacity Building and Strategic Partnerships for Chemicals Safety in the Republic of Serbia”** which was implemented by the **Ministry of Agriculture & Environmental Protection** of the Republic of Serbia with support of **UNDP** and funded by “**SAICM Quick Start Programme Trust Fund**”, in collaboration with two NGO, **ALHem** and **WECF**. *ALHem is a NGO based in Belgrade, Serbia. Its goal is promotion of sustainable development, with focus on chemical safety management & REACH implementation.*

TU256
SciRAP: Reporting and evaluation methods for non-standard toxicity, ecotoxicity, and nanoecotoxicity studies
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry
There is a need to facilitate the use of non-standard studies in regulatory assessments of chemicals, and at the same time, studies show that ecotoxicity and toxicity studies published in peer-reviewed journals are deselection for regulatory purposes due to insufficient reporting. To aid in these two processes, evaluation and reporting guidance for toxicity, ecotoxicity, and nanoecotoxicity studies are provided free of charge through the Science in Risk Assessment and Policy (SciRAP) web tool (www.scirap.org). SciRAP helps risk assessors to perform systematic and transparent evaluations of studies intended for use in hazard and risk assessment of chemicals. The methods provide criteria for evaluation of the reliability (i.e. inherent quality) of the study, as well as the relevance (in relation to a specific assessment) of the study. To each criteria additional guidance material is provided. The evaluation result is presented in an excel-file which displays the results using color-coding and figures. SciRAP also present reporting recommendations for peer-review publications. This guidance instructs researchers how to produce studies with high reliability and high reproducibility, and thereby increasing the likelihood for inclusion in regulatory assessments of chemicals. Moreover, a stringent reporting of studies allows editors, reviewers and regulators to evaluate the available information in a more structured and transparent way.

TU257
Tox on Tap: Engaging the community in science
S.E. Crawford, Institute for Environmental Research, RWTH Aachen / Dept. of Environmental Analysis; C.R. Labarrere, University of Saskatchewan / Toxicology Centre; D. Green, University of Saskatchewan - Toxicology Centre / Toxicology; E. Maloney, University of Saskatchewan / Toxicology; L. D'Silva, University of Saskatchewan - Toxicology Centre / Toxicology
Tox on Tap (ToT) is a forum for the discussion of current work and interesting scientific issues. Our volunteer based organization run by students aims to demystify scientific research for the general public and empower non-scientists to more comfortably and accurately assess science and technology issues, particularly those that stem from toxicological impacts in the environment. ToT is based on the French Cafe Philosophique model, Café Scientifique. It is a place where anyone can come to explore the latest ideas in science. Originating in England, the concept quickly gained popularity and was taken to other countries. Similar but independent events have arisen in many cities using variations of the Café Sci or Science Café name. Meetings have taken place in cafes, bars, restaurants and even theatres, but always outside a traditional academic context. The events are known for their informal and friendly atmosphere, and are believed to improve the image of scientists and careers in science. ToT organizes approximately 4-8 talks/events per year with speakers invited to start discussion on hot topics in toxicological science that are hosted in a local pub. Topics have ranged from lead, hunting and wildlife to aboriginal peoples and natural resources. We would like to share our stories and success in organizing these events in hopes that others are able to communicate their science to the public in a friendly and educational fashion.

TU258
Opening the black box: decision support systems and data mining for addressing uncertainty in ecological risk assessment
A. Hunka, Halmstad University / School of Business, Engineering and Science; S. Nowaczyk, S. Pashami, S. Waara, Halmstad University
Growing pressure on the environment, increasing complexity of regulations and substantial amounts of data to process during the ecological risk assessment (ERA) process contribute to making uncertainty an issue of significant concern. New methods, such as mechanistic models, have been introduced [1] in higher-tier

scenarios of ERA in the attempt to reduce complexity and increase ecological relevance, but very often predictive models are perceived as black boxes, increasing uncertainty in decision making instead of reducing it [2]. We postulate that in order to have ERA ready for emerging risks, it is not enough to develop more accurate predictions. The slow uptake of innovations, uncertainty-averse decisions and increasing complexity need to be systematically addressed first, to facilitate informed risk management and bridge the gap between academia and ERA practitioners. Our interdisciplinary group integrates computer science, environmental science and psychology, to systematically explore ways of advancing ERA decision-making with automated decision support systems. Data-driven approaches such as decision trees, Bayesian Networks or Deep Learning can help decision makers by increasing the effectiveness of information processing. In turn, this will lead towards more consistent decision making, since actual importance of various factors can be quantified. For example, when previous decisions and the factors that affected them are gathered in a knowledge base, created automatically from the data, it is possible to identify relevant differences between cases, and explicitly consider importance of the factors with respect to the decision. Data mining systems can predict the decision outcome and provide the confidence level for such outcome; however, additional research is needed on how to represent the resulting knowledge in an interpretable way. Our preliminary results with building scenarios based on past ERA rulings at the European level indicate that machine learning approaches can be successfully used in ERA to build trust and reduce uncertainty in decision making. [1]EFSA PPR Panel. 2014. Scientific Opinion on good modelling practice in the context of mechanistic effect models for risk assessment of plant protection products. *EFSA J* 12:92. [2]Hunka AD, Meli M, Thit A, Palmqvist A, Thorbek P, Forbes VE. 2013. Stakeholders' perspective on ecological modeling in environmental risk assessment of pesticides: challenges and opportunities. *Risk Anal* 33:68-79.

TU259
Institutional entrepreneurship for advancing toxicogenomics in ecological risk assessment: an interdisciplinary approach to bridging the gap between scientists and regulators
S. Maguire, McGill University / Desautels Faculty of Management; G. Hickey, McGill University - Macdonald Campus / Natural Resource Sciences; N. Basu, McGill University / Department of Environmental Health Sciences; D.E. Crump, Environment Canada; M. Hecker, University of Saskatchewan / School of the Environment and Sustainability and Toxicology Centre
Chemical contamination of ecosystems is one of the planet's greatest threats. Regulators and industry are tasked with assessing risks of chemicals – an enormous challenge given the number requiring evaluation, estimated at >100,000 worldwide. Current testing strategies typically rely on whole animal studies, which, in addition to ethical concerns, are associated with prohibitive time and monetary costs. There is, therefore, an urgent and recognized need for faster, focused, ethical, and efficient testing tools in regulatory ecotoxicology. This poster presents a unique interdisciplinary approach to advancing the use of toxicogenomics in ecological risk assessment. Bringing together ecotoxicologists with social scientists, regulators and industry actors, it aims to critically analyze what can be done to obtain a higher acceptability of toxicogenomic tools by regulators, firms, NGOs and other stakeholders. It does so by drawing on an established social science literature on "institutional entrepreneurship", i.e. "the activities of actors who have an interest in particular institutional arrangements and who leverage resources to create new institutions or to transform existing ones". Ecological risk assessment is a highly institutionalized field of professional practice. Methods used by risk assessors – and widely accepted by regulators and industry actors – are largely unquestioned and taken for granted, even if practitioners recognize shortcomings. Such entrenched practices are difficult to change as they are supported by what social scientists refer to as three institutional 'pillars': cognitive (i.e. actors think through, and construct reality with, existing practices), normative (i.e. actors place a high value on complying with existing practices), and regulative (i.e. professional and organizational reward systems create material disincentives for experimenting and innovating). Implementing change in such contexts presents significant challenges. Despite these difficulties, change can and does occur through "institutional entrepreneurship". Drawing on this analytical lens, we explore how regulators might benefit from toxicogenomics tools, and discuss challenges associated with adopting this technology in Canada and the USA. Preliminary findings from an ongoing research project bringing together academic, government and industry partners seeking to advance toxicogenomic solutions for ecological risk assessment are also presented.

TU260
SETAC Interest Group on Science and Risk Communication
T. Seiler, RWTH Aachen University / Ecosystem Analysis

TU261
SETAC Interest Group on Indigenous Knowledge and Values
R. Smith, Hydrobiology Pty Ltd

Advancing science and application of planetary boundaries and related ecological limits concepts to enable absolute sustainability assessments (P)

TU262
Ecological Public Health and DPSEEA – Tools towards the understanding of planetary health
G. Morris, S. Reis, T. Taylor, University of Exeter / European Centre for Environment and Human Health; L. Fleming, University of Exeter Medical School / European Centre for Environment and Human Health
The interconnections between environment and human health, and the notion of "planetary health," require the use of new tools to identify relationships, find solutions, and avoid unintended consequences of policies and actions. Bringing together the concepts of "ecosystem services" and "ecological public health," we attempt to provide the basis for discussion and analysis of complex environment-health issues, and create a framework for effective action towards improving planetary health. Conceptual models are useful tools with which to think, assemble information, communicate, and secure sectoral collaboration. We can use these models to effectively think through both the risks and benefits of human-environment interactions. Building initially on the World Health Organisation's DPSEEA Model (Corvalan 1996), a generic representation linking environmental "states" and their determinants to individual "exposure" and health "effects," the modified DPSEEA model (Morris, 2006) sought to represent greater social complexity in the relationship between environment and human health, including the health-promoting potential of environment. Subsequently, the ecosystems enriched (eDPSEEA) model (Reis 2015) explicitly recognised multiple routes to health and wellbeing, both through direct interactions and through ecosystem services, thusintegrating Health with Environmental Impact Assessment. The eDPSEEA approach takes the concept of Ecological Public Health (Rayner and Lang 2012) to an operational applied level. This is based on a recognition that the dynamic interactions between human and ecosystem health may take place distal in time and/or space, e.g. due to global climate. Ecological public health makes an imperative of a "quadruple bottom line" which delivers the environment, health, equity and sustainability; and in this context, resonates with the implied connectivity of the Sustainable Development Goals. Modelling within the Horizon 2020 funded INHERIT Project (http://www.inherit.eu/) expands on DPSEEA by highlighting the policy-relevance of the inter-relationship between individual agency and social level structures central to securing the lifestyle and behavioural changes necessary to deliver Ecological Public Health. Adding to this, economic tools may allow for the valuation of both ecosystem services and public health impacts, in order that policy makers can be better informed in designing policies to improve planetary health in a more holistic manner. **Acknowledgments** Funding provided by Horizon 2020

TU263
Towards understanding the environment's carrying capacity of chemical pollutants
A. Sobek, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; E. Gorokhova, Stockholm University / Department of Applied Environmental Science and Analytical Chemistry ACES
Planetary boundaries define a safe operating space for humanity. The current vision is that beyond these boundaries, vital Earth system processes may be negatively and irreversibly affected. However, no quantitative definition of the boundary for chemical pollution has been provided so far. In fact, chemical pollution may affect several global-scale boundaries involving biodiversity and nutrient cycles. Given the omnipresence of innumerable, and ever increasing, synthetic chemicals in the environment, the overarching objective of this project is to create new knowledge of the ecological mechanisms behind chemical-induced disruption of vital Earth system functions. Conventional toxicity assessments are limited to the effects of single or few chemicals on a few standard species, and are of little use to assess the effects of chemical pollution on essential ecological functions. We propose to apply the concept of chemical activity as a measure of chemical pollution and to study responses of microbial communities in the environments with varying chemical activity. The ubiquitous and ecologically essential functions that will be addressed are denitrification and ammonium oxidation that are responsible for essential processes in the planetary nitrogen cycle. First, we will use microbial denitrification as model system to establish a functional response to the chemical activity in sediment microcosms dosed with chemicals (chemical activity range: 0.01-0.1). The denitrification capacity will be assessed by quantitative PCR. Second, the model system will be expanded to include ammonium oxidation. Based on their phylogenetic origin and occurrence of functional genes, these vital functions are expected to have different resilience in response to chemical pollution. Finally, we will investigate the use of space-for-time substitution to detect Early Warning Signals of reduced resilience in microbial community functions along spatial gradients of chemical pollution.

TU264
Searching for the right number: which are the most suitable reference values to be compared to the planetary boundaries?

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A crucial point in the discussion around planetary boundaries is usually related to the difficulties and the uncertainties in defining a boundary, given the underpinning ecological and environmental complexity inherent in the evaluation. However, if those boundaries should be set in order to compare current level of pressure on environment and ecosystem with a reference value representing a threshold, another aspect become as well critical. In fact, the definition of the level of pressure may be also difficult as it is usually the results of accounting of emissions (often incomplete) or of modelling exercise (with the clear limitations that any modelling effort may entail). In order to monitor progress towards the goal of decoupling economic growth from the use of resources and their environmental impacts, the Joint Research Centre (JRC) of the European Commission (EC) has developed a framework and a life cycle based methodology , to assess the environmental impact associated to consumption and to calculate references, namely normalisation factors for life cycle assessment (LCA). The present study aims at shading light on the different options for assessing the level of environmental pressure and impacts, adopting life cycle impact assessment models for estimating the impacts. The study builds on the calculation of different sets of normalisation factors applicable in the LCA context and explore how those references look like when compared to planetary boundaries.

TU265

Could Planetary Boundaries be used as a basis for normalization in Life Cycle Assessment?

H. Teulon, Gingko21; C. Catalan, I Care & consult; J. Garcia, SCORE LCA; P. Osset, Solinnen
The growing concern around sustainability and environmental impact of products has been a driver for the development of methodologies to quantify such impact since the 1990s. Life Cycle Assessment (LCA) is a scientific approach recognized around the world to evaluate and identify the potential environmental impacts of a product or service over its lifecycle. However, interpretation of results is still difficult and it is too often submitted to value judgment. As a result, LCA fails to be adopted by decision makers as a reference tool for building their business strategy. According to ISO 14040-44 standard series, life cycle impact assessment is aimed at evaluating the significance of potential environmental impacts regarding different impact categories. It provides absolute values for different impact category indicators. It may be completed by a normalization step, which aims at calculating the magnitude of impact category indicators relative to reference information (usually compared with the total impacts in the region of interest). However, normalization does not provide information on the compatibility of the product under study with keeping human activities within acceptable ecological thresholds. The planetary boundaries concept was first proposed in 2009 and has recently been recognized as an interesting conceptual framework for defining potential ecological constraints or limits. However, applying the concept at the level of a company or a product raises a number of questions regarding methodology and practical applications. In order to answer some of these questions, SCORE LCA (a collaborative research organization dedicated to Life Cycle Assessment and environmental evaluation) launched a project on the application of Planetary Boundaries within LCA. The aim of this project is first to review existing literature on the topic, then to identify methodological issues and potential applications in long-term strategies of companies, and finally to test planetary boundaries as a basis for normalizing LCA results. The selected case study is a comparative assessment between thermal and electric cars in France in 2012 and 2020. \n *Keywords*—environmental boundaries, planetary boundaries, life cycle assessment, normalization \n

TU266

Key challenges for developing a Planetary Boundaries based life-cycle impact assessment methodology

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Life-Cycle Assessment (LCA) is a tool to quantify potential impacts of human activities to identify the environmentally better performing activity relative to other activities fulfilling the same function. However, the need for a shift from these relative assessments towards absolute assessments, where activities are related to sustainable levels of impacts, have been called for in both academia and industry. This study presents the key challenges related to developing a Planetary Boundaries (PB) based life-cycle impact assessment methodology (PB-LCIA). Indeed, a PB-LCIA allows for assessing the environmental performance of human activities relative to sustainable levels of impact as constrained by the PBs. The main benefits of a PB-LCIA are that impact scores are expressed in the same units as the PBs and that impact scores can be related to the PBs. We identified a number of challenges that should be addressed before a PB-LCIA can be developed and applied in practice. The challenges can be divided into two main categories. In the first category are challenges related to developing new characterization factors which express impact scores in the units of the control variables in the PB-framework. In the second category are challenges on how to use and interpret the results of the PB-LCIA. For instance, how to deal with the difference in principles on which LCA and the PB-framework are based, for instance the PB-framework is about keeping the Earth in a Holocene-like state while LCA is about protecting the areas of protection, i.e. humans, resources and the environment. Another example of a challenge related to interpretation of results is how to allocate the safe operating space to the activity being assessed. This is important because relating impact scores against the PBs, i.e. the full safe operating space, will not reveal if the activity is sustainable as no activity is on its own expected to exceed the safe operating space. Although a number of challenges have been identified, the new approach where environmental performance of activities is related to absolute boundaries can provide new, potentially valuable insights about the absolute sustainability of the assessed activities. These insights are expected to help decision-makers in not only indicating if an activity is environmentally better but also if it is environmentally good enough.

TU267

Position of existing footprints in the environmental sustainability landscape

A. Laurent, Technical University of Denmark / Division for Quantitative Sustainability Assessment, DTU Management Engineering; M. Owsianiak, Technical University of Denmark
To address the sustainability challenges, an increasing number of footprints have been developed. Their relative simplicity has contributed to make some of them, like ecological footprint and carbon footprint, widely used and broadly accepted by stakeholders in industry and authorities. But how do these different footprints relate to the overall context of environmental sustainability, and where are they positioned compared to other tools and framework such as life cycle assessment (LCA) and planetary boundaries (PB)? In this study, we performed a review of existing footprints, evaluated their roles and limitations in relation to more broadly-encompassing assessment tools like LCA, and discussed the need and approaches to set up footprint thresholds that can indicate levels, above which the analysed system reaches environmentally-unsustainable states, and help benchmark footprint results. Our results showed that the role of footprints should be detached from that of LCA, which aims to comprehensively assess environmental impacts while footprints are directed to addressing specific area of concern defined by the interest of society. Studies have showed that stand-alone footprints do not comprehensively capture all environmental problems, hence emphasising the need for researchers and stakeholders to be careful not to overstate the conclusions of their footprint results, e.g. claiming environmental sustainability when only performing a carbon footprint. To complement footprint results and facilitate interpretation and communication of the results to stakeholders, footprint thresholds are required. Global thresholds have been proposed in earlier studies. However, only in the case of blue water scarcity footprint did we find a good match between the PB framework and existing footprint thresholds. For carbon footprint, thresholds based on the 2-degree target were not found to completely match with the planetary boundaries for climate change, defined at the level preventing the Earth System from moving out of its stable, Holocene-like state. For other footprints, weak or irrelevant linkages between the footprint indicators and the PB were observed, e.g. ecological footprints. In this setting, we therefore recommend that science-based sustainability targets be consistently developed as footprint thresholds, also addressing the need to define these at scales corresponding to the assessed entities, e.g. individuals, products, organisations, industrial sectors.

TU268

Positive LCA Factoring Planetary Boundaries

D.G. Jones, Ecquate Pty Ltd / The Evah Institute; M. Vlieg, The Evah Intitute / Vlieg LCA; S. Ashar, Global GreenTag
If LCA is to assess architecturally ‘Positive Development’ in ‘eco-retrofitting of the vast urban fabric we already inhabit’ it must evolve beyond LCIA [1]. The authors extend life cycle assessment (LCA) beyond impact assessment (LCIA) to consider benefit assessment (LCBA) integrating planetary boundaries [2]. International Standard Organisation Environmental Management LCA methodology focuses mostly on pollution generation and resource depletion. The reach of LCIA is negative to zero often in loss of human health, ecosystem quality and natural capital. Apart from lower ecological loads LCA methods need to quantify recovery and regeneration of safe operating space and planetary boundaries for sustainable development. New concepts, methods and metrics to assess positive benefits, goals and benchmarks are needed to quantify positive outcomes. The proposed LCBA theory, methods and metrics supplement established LCIA methodology. Preliminary results are shown of a residential high rise garbage chute over 60 years use cradle to grave. Overall gains in space and diverting recyclables from landfill are far greater than losses from manufacturing impacts. Table 1 lists a few examples of the proposed LCBA schema to quantify gains. **Table 1 LCBA Schema Benefit Layer Positive Outcomes Hale Human Health Years Indoor Oxygen Oxygen generation with carcinogen sequestration Climate Braking Carbon sequestration for safer climate with less damage Positive Ecosystem Replenished Formation Urban Bounty Urban land area converted to full natural carrying capacity Stock Aquatic Restock marine catchment fauna &**

flora range biomass O_2 Supply Energy & Resource Viability Viable Supply Replenish concentrated & locally accessible resources Viable Water Replenish concentrated & locally accessible reservoirs References [1] Birkeland, J. (2008) Positive Development: From Vicious Circles to Virtuous Cycles etc,Earthscan 408 p. [2] Rockström, J., W. Steffen,. et al. 2009. Planetary boundaries: exploring the safe operating space for humanity. *Ecology and Society* <strong>14(2): 32. http://www.ecologyandsociety.org/vol14/iss2/art32/ October 2016 \n

TU269
Absolute sustainability assessment of the pressures exerted on biodiversity by the food portfolio of a retailer
A. Wolff, Mines Saint Etienne / Environmental Sciences; N. Gondran, Ecole des Mines Saint-Etienne / Génie de lEnvironnement et des Organisations; C. Brodhag, Ecole des Mines Saint-Etienne / Management responsable et innovation
Context: We are currently facing a dramatic decline of biodiversity. Governance institutions, such as the Convention for Biological Diversity, are calling the private sector to take actions (Nagoya, October 2010). Beyond local impacts on a company's land and premises, businesses can exert ecological pressures at multiple stages of their value chain through their operations, supply chains and the products and services they deliver. There is thus a need to develop approaches that assess businesses impacts in a holistic way and demonstrate whether the pressures exerted on biodiversity by a company are sustainable or not. Objective: This poster will present a method to assess the sustainability of the pressures exerted by an organization on biodiversity based on ecological boundaries. This approach will be illustrated based on the case study of a retail company with a focus on the pressures exerted at the agricultural production step. Materials and methods: We built on the developments of (Bjørn and Hauschild, 2015) who proposed a method of normalization of LCA midpoint impact categories based on ecological carrying capacities, that appear to be relevant for biodiversity conservation. In total, 880 categories of food products, representing more than 95% of the total mass of the food portfolio, were analyzed. We used the LCI database Agribalyse to estimate the impacts of the agricultural products at the perimeter cradle-to-farm-gate. The midpoint indicators were subsequently normalized with normalization references values proposed by (Bjørn and Hauschild, 2015) to estimate "ecological budgets" expressed in unit of person.year that can be interpreted as the "environmental interference corresponding to the annual personal share of the carrying capacity" (Bjørn et al., 2015). Results: Our results suggest that several pressures driving biodiversity loss are not sustainable. Indeed, some normalized pressures associated with food production exceed the market share of the retailer, which means that it occupies more ecological budgets than its consumers have, while these consumers cannot fully allocate their ecological budgets to food. Our results also highlight the prominent weigh of a few categories of product in the ecological burden. Discussion: Our results have implication for businesses as it suggests that the food sector still has a long way to go to lower its impacts down to levels that could be sustainable and compatible with biodiversity conservation.

Bridging between ecology, ecotoxicology and ecosystems services (P)

TU270
Novel Cell Reproductive Patterns in the Green Alga Pseudokirchneriella subcapitata and their Variations under Exposure to the Typical Toxicants Potassium Dichromate and 3,5-DCP
T. Yamagishi, N. Tatarazako, National Institute for Environmental Studies
Pseudokirchneriella subcapitata, formerly known as *Selenastrum capricornutum* or *Raphidocelis subcapitata*, is a sickle-shaped freshwater green microalga that is normally found in unicellular form. Currently, it is the best known and most frequently used ecotoxicological bioindicator species because of its high growth rate, sensitivity to toxicants, and good reproducibility compared with those of other algae. However, despite this organism's high notability, our knowledge of its cell biology—such as the patterns of nuclear and cytoplasmic division in the mitotic stage—is limited. It has been reported that *P. subcapitata* proliferates by forming four daughter cells (autospores) through multiple fission after two nuclear divisions by visualization of nuclei. Subsequently, the four autospores are typically released through rupture of the parental cell wall. Therefore, unlike the freshwater green microalga *Scenedesmus*, *P. subcapitata* does not form colonies. However, it is unclear how cytokinesis is achieved in the multinucleated cells containing four nuclei before autospore release.\n Here, we newly found two additional cell reproductive patterns in *P. subcapitata* apart from that by which four autospores are formed: cell reproduction by the formation of two autospores by binary fission ("two-autospore type") and cell reproduction by the formation of eight autospores by multiple fission after three nuclear divisions ("eight-autospore type"). By staining the nuclei of *P. subcapitata* with 4¢,6-diamidino-2-phenylindole (DAPI), we observed, in detail, nuclear and cytoplasmic division in cells cultured in OECD medium. In addition, we evaluated the frequency distributions of the different cell reproductive patterns from 24 to 120 h after the initiation of exposure to potassium dichromate ($K_2Cr_2O_7$) or 3,5-dichlorophenol (3,5-DCP), both of which are commonly used as toxicants in interlaboratory comparisons of algal toxicity testing. To our knowledge, this is the first reported detailed evaluation of the reproductive patterns of *P. subcapitata*, which changed dramatically in the presence of toxicants. These findings suggest that observation of the reproductive patterns of *P. subcapitata* may help to elucidate different cell reactions to toxicants. Finally, we discuss the relationship between cell size and cell reproduction patterns in *P. subcapitata*.

TU271
Searching for key parameters to increase the growth of Glyceria maxima under laboratory conditions
G. Gonsior, Eurofins Agroscience Services Ecotox GmbH / Aquatic Ecotoxicology
A ring test with *Glyceria maxima* is currently being discussed. In contrast to M*yriophyllum spicatum, Glyceria maxima* is a semi-aquatic species which has resulted in adaption of the recent guidelines and will therefore most likely require a new guideline to be written. Initial tests designs with *Glyceria maxima* have therefore been developed. One result of these tests was that *Gylceria* demonstrated different growth rates between different labs and also when comparing different tests conducted in a single lab. Variations between culture sources are a possible explanation for this result. Furthermore, plants can be generated from seeds or from rhizomes, resulting in different forms of the plant material. However, first test results indicate that these are not the only reasons for variability. We could demonstrate that the size of the plant material at test start also leads to different growth rates. It could not yet be clarified if seasonal variations also influence lab cultures. Lastly, the demands of nutrients, temperature and lighting regime required for *Gylceria* might be different when compared to *Myriophyllum*. Data will be presented with the focus on key parameters to increase growth of *Glyceria maxima* under lab conditions.

TU272
Aquatic plants: How to deal with different forms and consequences on endpoints?
G. Gonsior, Eurofins Agroscience Services Ecotox GmbH / Aquatic Ecotoxicology
During the evaluation of the OECD 239: "Water-sediment *Myriophyllum spicatum* Toxicity Test" the test species of interest changed from *Myriophyllum aquaticum* to *Myriophyllum spicatum*. Some publications suggested this change occurred because *M. aquaticum* was tested with the emerged form under submerged conditions. Due to this, not all effects observed could be related directly to the test item. Morphological adaptions of the plants to the submerged conditions influence test results. Further findings resulted in a discussion of the sensitivity of different forms of *M. aquaticum*. However, *M. aquaticum* is only one plant species which showed different forms of growth. Some aquatic plant species are able to grow on land, semi-aquatic and as completely submerged forms. Other species are able to produce underwater leaves, floating leaves and leaves growing out of the water. These leaves have different morphological and physiological functions. Therefore it is likely that the different forms of the same aquatic plant species used in tests have an influence on the outcome of the study data. Due to a decision from the EFSA (European Food Safety Authority) further aquatic plant species should be tested for the risk assessment of growth regulators and herbicides. In this context, data will be presented with the focus on adaption of test species for laboratory testing with realistic exposure scenarios.

TU273
What is the influence of natural dissolved organic matter on herbicide toxicity to two marine microalgae?
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Microalgae, as primary producers at the basis of aquatic food webs, can be indirect targets of herbicides. In their environment, these organisms are under the influence of other elements such as the dissolved organic matter (DOM), which can also interact with herbicides. Indeed, DOM can affect pollutant transport, fate, biodegradation, bioavailability, and toxicity on organisms. Therefore, the present study aimed to investigate the possible influence of natural DOM on the toxicity of three herbicides (irgarol (I), diuron (D) and S-metolachlor (S)) on two marine microalgae, *Chaetoceros calcitrans* and *Tetraselmis suecica*. The microalgae were exposed during 6 days, in triplicates, to each herbicide alone (I0.05 and I0.5 $\mu g.L^{-1}$; D0.05 and D0.5 $\mu g.L^{-1}$; S0.5 and S5 $\mu g.L^{-1}$) and to mixtures (M1: I0.05+D0.05+S0.5 and M2: I0.5+D0.5+S5). Experiments were run with and without DOM. The effects were assessed on growth rate (μ), photosynthetic efficiency (Φ'_M), and relative lipid content ($FL1_{lipids}$). The biological and chemical environments (bacteria, herbicide concentrations, DOM quantity and quality) were characterized. When no DOM was added to the cultures, the exposure of *C. calcitrans* to I0.5 and M2 significantly decreased μ and $FL1_{lipids}$ each at similar levels (around -55% and -50%, respectively) and Φ'_M (around -30%), as compared with controls. With DOM addition, lower significant effects were detected on $FL1_{lipids}$ (-25%) when the effects on μ and Φ'_M were almost the same (about -50% and -26%, respectively). For *T. suecica* without DOM, I0.5 and M2 significantly decreased the three biological parameters with M2 effects being significantly more

important than I0.5 effects (-63% vs. -56% on μ). When DOM was added, contrary to *C. calcitrans*, the observed effects were enhanced on μ (a decrease of -64% for I0.5 and -75% for M2) and Φ'_M while the effects on $FL1_{lipids}$ were decreased. These results may partly be explained by a possible complexation between DOM and herbicides, leading to a lower bioavailability of herbicides. However, the different responses of the two species to DOM addition remain to be explained. Quantitative and qualitative analyses of DOM (currently ongoing) and chemical analysis of herbicides concentrations will improve the understanding of these biological results, which show that DOM could possibly be involved in toxicity modulation in the environment.

TU274
The effects of two different types of agricultural land use on aquatic macroinvertebrate and algal communities
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The structure and functioning of freshwater communities are affected by changes in habitat quality resulting from how adjacent land is managed. In agricultural catchments, the two major land uses, arable and pastoral, differ in their management (e.g. ploughing, fertilizer and pesticide inputs) and therefore in their potential effect on freshwater communities. In this study we compared macroinvertebrate and algal communities in ponds and streams located in arable and pastoral dominated catchments. Twenty four sites were sampled (6 arable ponds, 6 arable streams, 6 pastoral ponds and 6 pastoral streams) and ecological communities in both streams and ponds were affected by land use. However, whereas the main effect on stream communities was on algal biomass and macroinvertebrates abundance and diversity, for pond communities the main effect was on algal biomass and community structure and on leaf litter processing. Arable streams had higher algal biomass and lower macroinvertebrate abundance and diversity than pastoral streams. Arable ponds had higher leaf litter processing, higher algal biomass and lower algal diversity, evenness, taxonomic richness and trophic diatom index, than pastoral ponds. Agricultural land use can adversely affect the structure and functioning of aquatic communities. Consequently, it may have a considerable potential impact on ecosystem services provided by freshwater habitats. Understanding the possible effects of agricultural land use on the structure and functioning of freshwater ecosystems is extremely important and should help in identifying the best land use management to maintain sustainable agricultural production and protect freshwater habitats.

TU275
Transcriptomic approach in microalga and macrophyte in-situ exposed in river impacted by chlor-alkali plant effluents
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Water quality degradation due to low concentrations of pollutants is a worldwide problem, but risk evaluation of chronic pollution *in-situ* is still a challenge. The present study aimed to evaluate the potential of transcriptomic analyses in representative aquatic primary producers to assess the impact of environmental pollution *in-situ*: the microalga *Chlamydomonas reinhardtii* and the macrophyte *Elodea nuttallii* were exposed 2hin the Babeni reservoir of the Olt River impacted by chlor-alkali plant effluents resulting in increased concentrations of Hg and NaCl in water. The response at the transcriptomic level was huge, resulting in up to 5'485, and 8'700 dysregulated genes (DG) for the microalga and for the macrophyte exposed in the most contaminated site, respectively. Transcriptomic response was congruent with the concentrations of Hg and NaCl in water in the Babeni reservoir affected by chlor-alkali plant effluents. Genes involved in development, energy metabolism, lipid metabolism, nutrition and RedOx homeostasis were affected by the *in-situ* exposure of both organisms. In addition, *C. reinhardtii* was affected for genes involved in the cell motility, while *E. nuttallii* was impacted for those involved in the development of the cell wall. DG were in line with adverse outcome pathways and transcriptomic studies reported after exposure to high concentrations of Hg and NaCl under controlled conditions in the laboratory. Transcriptomic response provided a sensitive measurement of the exposure as well as hints on the tolerance mechanisms of environmental pollution, and is thus promising as a more sensitive and an early-warning tool to assess water quality degradation.

TU276
SETAC Ecosystem Services Interest Group
S.E. Apitz, SEA Environmental Decisions Ltd

21st century Ecotoxicology and Human toxicology: Applications and perspectives for the use of OMICs data (P)

WE001
Can living organisms be replaced on enzymes in ecotoxicology?
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Fundamental scientific problem is the problem of monitoring a variety of processes that have a direct connection to the pollution of the environment, such as the environmental safety and quality control of the water, soil and air. The solution of this problem is important not only for all economic activities, but also it has significant importance in agricultural production and productivity in crop production. The searching of integral characteristics, which allow to assess the consequences of human impact on water, soil and air are very important. It should be noted the lack of developed methodology for integrated monitoring in the world practice that would help to solve the problem of integrated monitoring of the ecosystems. It was observed the trends of commonly use individual bioassays, which make wrong empirical results about the state of the environment and its influence on the individual as the main person of the biosphere. Another reason for getting wrong results during the bioassay analyzes is use living organisms and cultures, which can give a large measurement error. The proposed new approach and methodology of biochemical design of a set of enzymatic bioassays are the basis for the all-round bioassay called "enzymatic functional model of the living organism"; the development of scientific bases of such biochemical design could help to solve mentioned problems. The development of principles and mechanisms of a new methodology for biochemical design and a new fast methods for the bioluminescent analysis, which are intended for inclusion in a comprehensive system of assessment of the toxicity of different ecological environments is described. The bioluminescent methods of analysis are applied to the integrated task of assessing the ecotoxicity. A new methodology for the development of new bioluminescent methods for evaluating toxicity named by us biochemical (or biotechnological) design have been shown. The algorithm of biotechnological design of the bioluminescent methods and reagents for environmental monitoring of the water, soil and air have been shown. Methodological approaches will be different, because it will be developed as rapid non-specific methods and as a selective analysis is based on the measurement of markers, the markers will be the substrates of bioluminescent reactions and the activity of enzymes involved in luciferase enzymatic chains. The research was supported by the Russian Science Foundation, project No 16-14-10115.

WE002
An integrative approach for understanding the adverse outcome pathways in algae: Method development towards anchoring molecular and apical endpoints
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Omics technologies can enable insights into changes of global molecular profiles induced by toxicant exposure. One of the challenges for integrating the generated data into formal chemical risk assessment is a demonstration of the robustness, accuracy and relevance of toxico-*omics* data. In this proof-of-concept study, we aim to employ multi-'*omics'* technologies (transcriptomics, metabolomics and lipidomics) to profile the molecular stress response of *Chlamydomonas reinhardtii* to toxic insult, and identify molecular key events predictive of phenotypic adverse outcome, using network construction of the gene-metabolite-adverse outcome interaction space and network inference of causal effect chains applying a weight-of-evidence approach. Our initial objectives, as presented in this poster presentation, were to adapt existing test protocols for *C. reinhardtii* to accommodate toxicokinetic properties of volatile chemicals while complying with OECD guideline 201 validity criteria on growth inhibition tests in algae (specific growth rate >0.92/d, pH drift < 1.5 over 72h), and to utilise the developed test system to investigate the toxicity of the baseline toxicant chlorobenzene. Algae were cultured in vials with no gas phase along varied inoculation densities, photoperiod duration and bicarbonate supplementation, and were exposed to a chlorobenzene concentration range to determine effective levels of growth inhibition. The developed rigorous testing system was validated with the toxicity reference 3,5-dichlorophenol and prescribes inoculation cell densities, photoperiod duration and bicarbonate supplementation levels effecting growth dynamics that fulfil OECD test validity criteria over a 72h test period. An EC_{50} of chlorobenzene for *C. reinhardtii* was designated. In the next step, the established test data will be employed to designate exposure concentration and time-point pairs for *C. reinhardtii* metabolite and RNA extraction to hypothesize on candidate molecular toxicological key events in the stress response to baseline toxicant exposure. Such an investigation will entail the development of a workflow for algal *omics*-phenotyping to advance the notion of hypothesis-free *omics*-driven AOP and key event discovery.

WE003
GEMTOX - predicting mixture toxicity from single chemical gene expression
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Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology
Before a chemical is released onto the market, companies have to provide information on its toxicity. Possible synergistic and antagonistic effects with other chemicals are not taken into account due to the high number of experiments that would be required. Therefore, an interest in methods that predict the joint effect of two or more chemicals based on single chemical experiments has emerged. The mixture toxicity models are simple and their accuracy is low. We want to improve upon these methods by introducing metabolic modeling in the form of Flux Balance Analysis(FBA) to ecotoxicology. FBA is a linear programming framework that allows the calculation of equilibrium metabolic reaction fluxes on a genome scale, based on the stoichiometry of the metabolic network and nutrient uptake rates. We want to predict mixture effects on growth by integrating gene expression as constraints into the FBA and then combining the effects that the chemicals have on different metabolic pathways. In the first phase of our research we are assessing the accuracy of FBA for quantitative toxicity predictions of single chemical exposure. Here, we present predictions of growth reduction in the green alga C. reinhardtii, upon exposure to silver ions and three herbicides.

WE004
Developing Confidence in Adverse Outcome Pathway-Based Toxicity Predictions -Effects of the Fungicide Imazalil on Fathead Minnow Reproduction
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An adverse outcome pathway (AOP) description linking inhibition of aromatase (cytochrome P450 [cyp] 19) to reproductive dysfunction was reviewed for scientific and technical quality and endorsed by the OECD (https://aopwiki.org/wiki/index.php/Aop:25). An intended application of the AOP framework is to support the use of mechanistic or pathway-based data to infer or predict chemical hazards and apical adverse outcomes. As part of this work, ToxCast high throughput screening data were used to identify a chemicals' ability to inhibit aromatase activity in vitro. Twenty-four hour in vivo exposures, focused on effects on production and circulating concentrations of 17β-estradiol (E2), key events in the AOP, were conducted to verify in vivo activity. Based on these results, imazalil was selected as a case study chemical to test an AOP-based hazard prediction. A computational model of the fish hypothalamic-pituitary-gonadal-liver axis and a statistically-based model of oocyte growth dynamics were used to predict impacts of different concentrations of imazalil on multiple key events along the AOP, assuming continuous exposure for 21 d. Results of the model simulations were used to select test concentrations and design a fathead minnow reproduction study in which fish were exposed to 20, 60, or 200 μg imazalil/L for durations of 2.5, 10, or 21d. Within 60 h of exposure, female fathead minnows showed significant reductions in ex vivo production of E2, circulating E2 concentrations, and significant increases in the ovarian expression of mRNA transcripts coding for cyp19a1a, cyp11a, and cyp17. A concentration-dependent decrease in cumulative fecundity was also detected for fathead minnow pairs exposed continuously for 21 d. Overall, results of the study provide strong support for the qualitative relationships represented in the AOP and provide further evidence of concentration-response and temporal concordance among key events. The quantitative models over-estimated the in vivo potency, suggesting that additional chemical-specific properties must be taken into consideration in order to reliably use this quantitative AOP construct for predictive risk assessment. *The contents of this presentation neither constitute nor necessarily reflect US EPA policy.*

WE005
Link of protein carbonylation and aging after chronic gamma irradiation of C. elegans glp-1 mutant
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Nature provided environmental ionizing radiations which can be overexpressed by nuclear accidents, *e.g.* Fukushima. Although in the first instance, gamma radiation effect and aging are both mainly mediated by reactive oxygen species, there is a lack of knowledge regarding the effects of chronic gamma irradiation on ecological endpoints such as lifespan. Classical radiobiological models focus on DNA damage, but it has been shown that proteome protection, mainly against protein oxidation (carbonylation), can be linked to radiosensitivity. Hence, we investigated the link between protein carbonylation and aging, with and without chronic gamma irradiation. The mutant *glp1 - Caenorhabditis elegans* has been used because of its sterility at 25°C which enables single generation study over its whole life cycle. Chronic irradiation has been conducted from eggs to either L4 or old stages, at 2 different dose rates to finally obtain the same cumulated dose for each irradiated group. Two irradiation designs were performed: group 1 – irradiation from eggs to L4 stage (3 days) with 50 mGy.h^{-1} reaching 3.3 Gy and group 2 - irradiation from eggs to "old" stage (19 days) with 7 mGy.h^{-1} reaching 3.3 Gy. Life span and protein carbonylation were followed in both groups. Protein carbonylation level was measured with *in situ* biomolecular assessment and the nature of carbonylated proteins was done with OxyDIGE methodology. Carbonylation level of the old stage worms is higher than the one of L4 stage worms. Furthermore, there is a tendency that carbonylation level decreases in both groups after chronic radiation exposure, with a significant difference found in group 2. Even without significant difference, the life span prolongation after 7 mGy.h^{-1} exposure for 19 days compared to control can indicate that low dose rate of irradiation created better antioxidant protection than 50 mGy.h^{-1} for 3 days compared to control. By studying classical oxidative damage to proteins, we found that all differential carbonylated proteins between radiation and control are involved in aging and/or energy metabolism in a sense to protect the irradiated worms. This result could participate in enhancing the knowledge about antioxidative contribution to lifespan extension. Key words: *C. elegans glp-1 mutant*, chronic gamma irradiation, aging, carbonylation

WE006
Proteome analysis of male gonads in Gammarus fossarum exposed to Pyriproxyfen: mining for endocrine disruption biomarkers
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Proteomics is becoming popular in ecotoxicology as an approach for the discovery of new candidate biomarkers of environmental contamination. Studies are currently based on the classical proteomic approach, in which proteomes from a control and a contaminated situation are resolved by 2D-PAGE, and proteins differentially expressed are then identified by mass spectrometry. Most of these detected proteins are abundant, usually housekeeping proteins, such as those involved in ATP metabolism and maintenance of the cytoskeletal structure, but rarely specific to a contamination exposure. This drawback is due to the few sequenced genomes in aquatic invertebrates that limit the interpretation of the mass spectra and the identification of proteins, and the low dynamic range of the 2D-PAGE approach. Recent technological advances in high-throughput "omics" methodologies, in which thousands of genes, proteins or metabolites can be assessed simultaneously, provide invaluable molecular information. Shotgun proteomics allows in-depth analysis of whole proteomes if performed with the most recent mass spectrometers. In order to unveil the novel perspectives offered by the latest generation of mass analyzer, we analyzed the performances of the Q-Exactive HF tandem mass spectrometer equipped with an ultra-high field Orbitrap analyzer. The aim of this study was to reconsider the discovery of new candidate biomarkers of exposure to Pyriproxyfen in male gammarids by shotgun proteomics, in the light of the latest generation of tandem mass spectrometer. Males were exposed to two concentrations of the xenobiotic Pyriproxyfen (0.5 and 50 μg/L) during two consecutive spermatogenesis cycles. The number of proteins identified, their abundance and the number of proteins that were modulated by the contamination were used to document the performance of the new generation mass spectrometers in the context of environmental ecotoxicology. The Q-Exactive HF allowed for the identification of a total of 2047 proteins. Amongst this large panel of proteins, a set of 32 were detected as significantly modulated by pyriproxyfen at 0.5 μg/L and 21 by pyriproxyfen at 50 μg/L. Among them the majority were related to catalytic and binding proteins.

WE007
Multigenerational effects of Daphnia magna exposed to organic contaminated stream water: A proteomic-global DNA methylation approach
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It is now well accepted fact that the multigenerational effects of chemicals should be incorporated in risk assessment. Herein, we report a comprehensive study with different phenotypical as well as physiological responses of *Daphnia magna* continuously cultured for three generations (F0, F1 & F2) in field water (PW) which was collected from surface stream water with possible industrial as well as natural contaminants. On one hand, increase in body size, spine length, reproduction rate, hemoglobin formation were observed, on the other hand, slower movement and decrease in heart rate were evident in PW-exposed animals. In addition, we found a possible physiological adaptation across generations. To find out the possible correlation of molecular mechanism and phenotypical phenomena, we conducted proteomics, behavior analysis as well as global DNA methylation status in all generations. In the F0 generation, all defense mechanisms were evoked, such as, increase in body size, spine length, metabolic activity, Hb conc., reproduction etc. Decrease in number of proteins, pathways, biological interactions proves prevalent adaptive mechanisms from F0 to F2. The S-adenosylmethionine synthase' (the enzyme to catalyze SAM protein formation) was up-regulated in F0-PW-exposed

animals, which might causes hypermethylation in PW-exposed organisms. In behavior analysis, we also found markedly significant difference between laboratory cultured (in M4) and PW-cultured daphnia in respect of speed, acceleration, locomotion, turning rate etc. Taken together, our results present a paradigm not only of multigenerational exposure-effect quantification but also exhibit another important layer of observation by combining OMICS-epigenetic-behavior in complex mixture stressors with real field water. \n Keyword: Multi-generational effects, Contaminated field water, Proteomics, *Daphnia magna*

WE008

High conservation in transcriptomic and proteomic response of white sturgeon to equipotent concentrations of 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene

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Adverse effects associated with exposure to dioxin-like compounds (DLCs) are mediated primarily through activation of the aryl hydrocarbon receptor (AHR). However, little is known about the cascades of events that link activation of the AHR to apical adverse effects. Therefore, this study used high-throughput, next-generation molecular tools to investigate similarities and differences in whole transcriptome and whole proteome responses to equipotent concentrations of three agonists of the AHR, 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene, in livers of a non-model fish, the white sturgeon (*Acipenser transmontanus*). A total of 926 and 658 unique transcripts were up- and down-regulated, respectively, by one or more of the three chemicals. Of the transcripts shared by responses to all three chemicals, 85% of up-regulated transcripts and 75% of down-regulated transcripts had the same magnitude of response. A total of 290 and 110 unique proteins were up- and down-regulated, respectively, by one or more of the three chemicals. Of the proteins shared by responses to all three chemicals, 70% of up-regulated proteins and 48% of down-regulated proteins had the same magnitude of response. Among treatments there was 68% similarity between the global transcriptome and global proteome. Pathway analysis revealed that perturbed physiological processes were indistinguishable between equipotent concentrations of the three chemicals. The results of this study contribute towards more completely describing adverse outcome pathways associated with activation of the AHR.

WE009

3-Step Analytical Methodology for the determination of antidepressants and antipsychotics in human hair by LC- hybrid LTQ Orbitrap MS

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In the present study a simple and sensitive method for the determination of seven antidepressants and antipsychotics (clozapine, olanzapine, haloperidol, mirtazapine, venlafaxine, amisulpride and carbamazepine) in human hair has been developed and validated. Human hair samples were collected from a healthy volunteer. Hair sample (0.1 g) was digested in NaOH (80 °C, 20 min) and subsequently pH was readjusted to 9.0-10.0 with HCl. Then, the sample was mixed and dispersed using Aluminum oxide and $MgSO_4$. The blend was transferred to a polypropylene cartridge and analytes were eluted sequentially with DCM, methanol/DCM (50:50) and methanol. Then, QuEChERS cleanup was performed: 1 mL of the extract was transferred to a centrifuge tube containing $MgSO_4$ and PSA. The tube was vigorously mixed for 1 min and centrifuged for 10 min to 4000 rpm. The supernatant was collected and evaporated till dryness. The residue was reconstituted in 100 μL methanol with 0.1% NH_3 and injected into the chromatographic system. Detection of the target analytes was performed using an LC- Hybrid LTQ –Orbitrap MS system in positive ionization mode in a total run of 10 min. The column we used was Hypersil Gold Column (50 x 2,1 mm, 1,9 μm) Thermo Scientific (Waltham, MA USA). The validation scheme followed was based on the US Food and Drug Administration FDA [1]. Good linearity was obtained in all cases exhibiting excellent coefficients of determination (R^2). The method precision achieved in terms of repeatability and within-lab reproducibility, was low enough, expressed as relative standard deviation (R.S.D.), complying with the requirements of US FDA document (< 8%). Recoveries obtained were satisfactory for all analytes (90-108 %) while limits of quantification found at the low ppb level (2,5-12,5 μg/kg). The ultimate purpose was the successful application in the field of hair analysis of psychiatric patients of different treatments and poste mortem samples. **ACKNOWLEDGEMENTS** The authors would like to thank the Unit of Environmental, Organic and Biochemical high resolution analysis-ORBITRAP-LC-MS of the University of Ioannina for providing access to the facilities. **REFERENCES** [1] FDA Guidance for Industry on Biomedical Method Validation, 2001, http://www.fda.gov/Drugs/default.htm

WE010

Cytotoxic and genotoxic effects of contaminants present in a highly polluted estuary in multiple organs of rats

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Estuaries are among the most pressured ecosystems due the contamination resulting from anthropogenic activities. Santos-São Vicente Estuary is located in Santos city, coastal region of Sao Paulo/Brazil. This estuarine area is highly contaminated with heavy metals, PAHs, detergents, etc. Estuaries also offer food for human consumption, resources for industries and areas for recreational activities, thus estuary contamination is a public health issue as well an environmental problem. The aim of this study was to analyze the cytotoxic and genotoxic potential of the contaminants presented in this estuary in multiple organs of rats. For this propose, surface water was collected in two polluted points (PA and PB). Wistar rats were distributed into four groups and exposed for five days to: *a)* negative controls received filtered water, *b)* saline controls received saline water, *c)* experimental group received estuarine water from PA and *d)* experimental group received estuarine water from PB. Then, animals were euthanized and liver and bone marrow were properly prepared to micronucleus and comet assays, and liver was prepared to histopathological analysis including the presence of apoptotic bodies, ki67 immunohistochemistry and PCR for caspase-3. Heavy metal concentration in water was analyzed by atomic absorption spectrometry. Heavy metal analyses indicate the presence of mercury, cadmium and arsenic at PA, and arsenic and lead at PB. There were significant increase in micronuclei frequency and DNA breakage in bone marrow and hepatocytes of rats exposed to estuarine water when compared to both controls (negative and saline). There were no histopathologic alterations and differences in the number of apoptotic bodies between the groups. Ki67 expression were greater in hepatocytes of rats exposed to estuarine water the control groups. Gene expression levels demonstrated a significantly decreases in expression of caspase-3 in experimental groups compared to negative control. The estuarine water is contaminated with substances that have harmful biological effects multiple organs of rats closely related to genotoxicity and cell cycle regulation process. Thus, mitigation policies of environmental impacts should be done in order to avoid future health problems. The authors thank the financial support of the National Research Council (CNPq).

WE011

iNVERTOX: Rapid intelligent in silico prediction of sub-lethal ecotoxicological effects in invertebrates following pharmaceutical exposure

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Occurrence of pharmaceuticals as environmental contaminants has been well reported in surface waters, sediments and biota in aquatic environments. These compounds may have adverse effects on reproduction, development and behaviour in biota.[1] However, current effect assessments fail to fully link the cause-effect relationships. Furthermore, pharmaceuticals, generally, show a low potential to accumulate. Thus, there is need to assess the effects of innate levels of pharmaceuticals found in aquatic biota. Previous investigations have found statistically significant changes associated with endogenous metabolites upon exposure of *Gammarus pulex* to three pharmaceutical compounds and personal care products (propranolol, nimesulide and triclosan)[2]. Herein, we present a novel approach that will aim to enable phenotypic anchoring of metabolomics data and internal pharmaceutical concentrations to physiological responses. With the sheer volume and complexity of the collected data, intelligent approaches that encompass machine learning methods such as artificial neural networks, support vector machines and tree-based learning will be investigated. Optimisation of *in silico* approaches will enable rapid interpretation of how pharmaceuticals interact with the metabolome and lead to physiological effects in aquatic invertebrates. Finally, computational models will also be used to predict the physiological responses of invertebrates upon exposure to pharmaceuticals. The iNVERTOX project will, for the first time, allow a comprehensive analysis of cause-effect relationships in aquatic organisms that have been exposed to pharmaceutical contaminants. Thus, enabling reliable and accurate risk assessment of the potential adverse effects related to pharmaceutical contamination in the aquatic environment. References. 1) Daughton, C.G. & Ternes, T.A. (1999). *Environ. Health. Persp.*, 107(6), 907-938. 2) Gómez-Canela, C., et al. (2016). *Sci. Total. Environ.,* 562: 777-788.

WE012

Molecular Toxicity of Metal Mixture and Defense Mechanisms in Zebrafish (Danio rerio).

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Metals are important components of environment and play a pivotal role in various biochemical and physiological processes. These are widely found in nature, particularly in mineral deposits and soils, meaning that they are available to be taken up by plants and animals that serve as a food source for humans. Virtually, all metals, including the essential metal micronutrients, are toxic to both fresh and marine aquatic organisms as well as humans if exposure levels are sufficiently high. The toxicity of the most important metals has been extensively documented for various species and the results are used to derive environmental standards. Most experimental studies on metal toxicity in aquatic systems consider the water phase as the main exposure route. However, food can also be an important source of metal exposure, but this route is often considered of little importance in relation to toxicity. The experimental evidence for this is rather limited and would imply that metal uptake via the digestive route is low and /or that the internal handling of the metals taken via food is different from uptake from the water phase. Recent research has raised significant concerns with respect to diet borne metals. Total metal exposure may be similar via ingestion or via the gills. Today the information on the potential metal – metal interaction within the intestinal lumen as well as the studies which investigate the change of gastrointestinal environment by a metal, its effect on the uptake of a subsequently ingested metal and potential toxicity of one on another is insufficient, also insufficient information on comparative study in different animal models is available. The focus of this work is to extensively understand toxicity mechanisms and gene expression in relation to the metal toxicity in Zebra fish and provide an insight into the working in other organisms, as well as identify similarity and differences. Further study will help to develop new strategy to combat metal toxicity. **Keywords**: Toxicity, Uptake, Diet borne, gene expression.

WE013
Quantitative relationships between the structure of pharmaceuticals and the expression of genes involved in liver damage
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In the recent years, increasing concerns about the potential harmful effects of compounds present in the environment has led to the fast development of technologies such as toxicogenomics and *in silico* modelling as effective support in hazard and risk assessment procedures. In addition, these approaches find valuable application in drug design and drug development procedures, to guarantee the safety of new pharmaceuticals. Among several projects started worldwide, the Toxicogenomics Project in Japan (TPJ), set up by the Ministry of Health, Labour and Welfare, National Institute of Health Sciences, and several pharmaceutical companies, published a large database (TG-GATEs), which included gene expression and pathology information for more than a hundred pharmaceuticals. Results from a recent study (Low et al. Chem Res Toxicol. 2011), which were based on data from the TPJ mentioned above, evidenced that Endoplasmic Reticulum stress after treatment with several hepatotoxicants may be involved in liver damage through phosphorylation of the eukaryotic initiation factor Eif2 signaling pathway. This mechanism involves deregulation of several genes, such as Eif2 subunit 1 alpha (*Eif2s1*), Eif3 subunits G (*Eif3G*) and J (*Eif3J*), and *Eif4a1.* In this study, we present preliminary results for the development of quantitative relationships between the structures of 120 heterogeneous pharmaceuticals included in the TPJ database and the expression of deregulated genes (i.e. *Eif2s1, Eif3G, Eif3J* and *Eif4a1*) after rat exposure to these compounds (24h and high dose). In particular, the differential expression of the four genes deregulated by phosphorylation of Eif2 induced by the endoplasmic reticulum stress, were used as activity responses in the development of Quantitative Structure-Activity Relationships (QSAR). Molecular descriptors were calculated *in silico* from the 3D structure of the 120 pharmaceuticals using the software Padel Descriptors v. 2.21. Six QSAR models were generated by applying multiple linear regression in the software QSARINS. The best combinations of modelling variables were chosen by the genetic algorithm variable selection procedure. The models were successfully validated internally, to verify their descriptive ability and robustness, and externally, to evaluate their predictive power. In addition, the possible relationship between gene expression and liver toxicity was investigated by multivariate analysis.

WE014
Influence of Toluene exposure on metabolomics responses of Oryza sativa (Asian rice)
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As frequency and scale of the chemical incidents have been increased, the ecological damage caused by the chemical incidents emerged as a serious problem in Korea. To estimate the extent of damage and the amount of compensation, the methodology for assessing ecological impact through field investigation was developed However, existing method for assessing the damage to plants and crops only relied on the change in their phenotype such as leaf-bronzing, so there has been lots of controversy because of inaccurate diagnosis technique and quantitative analysis. Therefore, metabolomics, which has emerged as a powerful tool for sensitive and early diagnosis, should be introduced for chemical accident damage assessment. The development potential of metabolomics-based damage diagnosis tool was studied using Oryza sativa (Asian rice). Toluene was selected as target compound based on the scoring system which takes into account both accident frequency and hazards. In this study, the metabolomics responses of Oryza sativa in hydroponic culture at early development stages (4th leaf stage) to toluene was evaluated by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-QToF-MS) based non-targeted metabolic profiling. The exposed concentration-based and recovery time-based metabolic response patterns were analyzed by Principal component analysis. In addition, candidate marker compounds for damage diagnosis were identified by drawing heat map and calculating variable important for projection (VIP) scores of partial least square discriminant analysis (PLSDA). Moreover, metabolic effect level index (MELI), which was developed for quantitative index in previous study, was applied to identify the applicability of readable endpoint. Overall, the results of these multivariate statistical analysis demonstrated a number of potential biomarkers that were characterized by metabolomic approach and provided an insight into quantitative chemical accident damage assessment. This subject is supported by Korea Ministry of Environment (MOE) as "The Chemical Accident Prevention Technology Development Project."

WE015
Reference genome of the freshwater snail Lymnaea stagnalis, a multidisciplinary invertebrate model
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The great pond snail *Lymnaea stagnalis* is a model organism in various research domains, including neurophysiology, evolution, and ecotoxicology. Regarding chemicals regulation, it is the model species of a new OECD test guideline (Test No. 243: *Lymnaea stagnalis* Reproduction Test). However, a reference genome was still lacking for this species. This situation motivated us to set up a multidisciplinary consortium, “lymnaea **STAG**nalis **IN**ternational **G**enome initiative” (STAGING). Taking advantage of the ability of this simultaneous hermaphrodite to self-fertilize, we sequenced a highly homozygous genotype obtained through several generations of alternating selfing and sib-mating. Whole genome shotgun sequencing of short fragments was performed with HiSeq2500 (2x250bp) and MiSeq (2x300bp) techniques (fragment size 400-700 bp). Scaffolding was based on the sequencing of mate-pair libraries (Nextera) of 3 to 15 kb insert sizes (Hiseq2000). In total, 6640 scaffolds of size > 2kb were obtained, with an assembly size of 943 Mb (N50 = 957 kb ; L50 = 297 contigs). Repeat sequence detection (tandem repeat, low complexity sequences) and annotation (transposable elements) were used for genome masking and further annotation. These sequences represent 34.6% of the total assembly (326 Mb). Exon/intron structure was inferred from gene model prediction based on ab initio and evidence-driven methods (ESTs, RNAseq data, protein mapping). Transcriptomic data obtained from Illumina and Nanopore technologies were combined to produce long and accurate synthetic reads. Genomic mapping identified 22499 genes (3492 without intron), with a mean/median size of 15299/8502 bp, and a mean/median number of exons of 7.59/5. A proportion of 56.4% of proteins conserved among Gastropoda mapped onto the assembly. Expert annotation is ongoing, focused on molecular pathways of interest to the Consortium. Regarding ecotoxicological issues, we particularly focus on molecular/cellular stress response and signaling pathways. Also, specific attention is given to nuclear receptors and potential targets of endocrine disruption. By the time of the meeting, the *L. stagnalis genome-browser* will be made public, providing a unique resource for scientific research and environmental issues involving this species and other molluscs. Acknowledgements – STAGING was funded by France Génomique (https://www.france-genomique.org/spip/spip.php?article144), and benefited from Anaee facilities (ANR-11-INBS-0001).

WE016
SETAC Omics Interest Group
B. Campos, IDAEA-CSIC / Environmental Chemistry

Environmental endocrine compound concentrations and human and ecosystem health effects (P)

WE017
Estrogenic activity of waterbodies with water bloom and the potential contribution of natural and anthropogenic compounds
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Compounds with estrogenic activity have been widely studied as they can have adverse effects on wildlife populations. They can be of anthropogenic as well as natural origin. Rivers and streams affected by waste water treatment plant effluents received the main attention and much less is known about other types of freshwaters. Therefore our study focused on still waters (ponds, reservoirs) and we assessed the presence and potential contribution of various types of estrogenic compounds to the overall estrogenic activity of these waters. During the summer in the years 2013 and 2014, we collected water samples from ponds and reservoirs with water blooms. In these samples, we measured concentrations of anthropogenic contaminants (estrogenic hormones, phenolic compounds) and natural substances (flavonoids and phytosterols) by LC-MS/MS. Estrogenic potencies of these compounds and the estrogenic activity of water samples were determined using the *in vitro* bioassay with the hERα-HeLa-9903 cell line. From the concentrations and estrogenic potencies, we were able to calculate the contribution of individual compounds to the estrogenic activity observed for water samples. Most of the water samples showed detectable estrogenic activity (detection limit 0.02 ng/l) with estrogen equivalents exceeding 1 ng/l in some cases. These levels indicate the potential for adverse impact on the aquatic life. Concentrations of estrogenic hormones and flavonoids were in the range of ng/l, phenolic compounds were detected in tens of ng/l. Phytosterols were present in concentrations up to several μg/l, but they failed to induce any estrogenic response in the tested range of concentrations (up to 6E-06 M; their estrogenic potencies were lower than 9E-07) and so they were not included in the calculations of contribution to the estrogenic activity of water samples. Estrogenic hormones were the most potent compounds and in a few cases they entirely explained observed activity of water samples. Flavonoids and phenolic compounds were much less potent and accounted for less than 9 % of the estrogenicity of water samples. However, the estrogenic equivalent of many samples was higher than could be anticipated from the chemical analyses and more than 90 % of the estrogenic activity could not be attributed to any of the analysed compounds. The work was supported by EU FP7 project SOLUTIONS (No. 603437) and by the Czech Ministry of Education, Youth and Sports (LO1214 and LM2015051).

WE018
Effects of endocrine disrupting chemicals (EDCs) to Caenorhabditis elegans and Folsomia candida
J. Moon, D. Kim, J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science
This study investigated the adverse effect of endocrine disrupting chemicals (EDCs) on soil organisms. Test EDCs includes Bisphenol A (BPA) and Bis(2-ethylhexyl) phthalate (DEHP), and test species were soil nematode (*Caenorhabditis elegans*) and springtail (*Folsomia candida*) that are standard test species for soil eco-toxicity test in ISO and OECD guidelines.The survival and reproduction assays for springtails were conducted following OECD guideline. Ten *F. candida* were exposed to BPA and DEHP amended LUFA 2.2 soil, and survival and offsprings numbers were observed. For soil nematode population toxicity test, we observed offsprings of *C. elegans*. In the result, $NOEC_{BPA.Rep}$ (BPA, reproduction) and $EC10_{BPA.Rep}$ of *F. candida* were 500 mg/kg dry soil and 191.4 mg/kg dry soil. $NOEC_{DEHP.Rep}$ and $EC10_{DEHP.Rep}$ of *F. candida* were < 10 mg/kg dry soil and 2.4 mg/kg dry soil. On the other hand, $LC50_{BPA}$ $LC50_{DEHP}$ of *F. candida* were 2655.2 and 2275 mg/kg dry soil respectively. In the soil population of soil nematode assays, $NOEC_{BPA.Rep}$ (BPA, reproduction) and $EC10_{BPA.Rep}$ of *C. elegans* were 100 mg/kg dry soil and 36.7 mg/kg dry soil. $NOEC_{DEHP.Rep}$ and $EC10_{DEHP.Rep}$ of *C. elegans* were 500 mg/kg dry soil and 12.2 mg/kg dry soil. This study can be preliminary toxicity data for ecological risk assessment of endocrine disrupting chemicals in soils. *This study was funded by the Korea Ministry of Environment (MOE) as the Environmental Health Action Program (RE201603033), and Graduate School of Specialization for managing information of chemical risk. \n*

WE019
Endocrine disruptors in soil: effects of Bisphenol A on growth, reproduction, immunity and gene expression in earthworms.
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The application of biosolids as organic fertilizers in agricultural fields is increasing. These residues used in agriculture contain not only nutrients but also xenobiotics, such as bisphenol A (BPA). These compounds of anthropogenic origin are not regulated by law on the use of biosolids in agriculture, which highlights the need to assess their toxicological effects on soil life, whose most abundant animal representatives are earthworms. In this study the effect of BPA on life history parameters such as growth and reproduction were evaluated for *Dendrobaena veneta* and *Eisenia fetida* earthworms. Moreover viability of coelomocytes and gene expression changes were tested. Sublethal concentrations of 1, 10, 100, 1000 and 2000 mg/kg were tested by the OECD artificial soil test after their selection in a preliminary study. Decline in growth at the two highest concentrations was detected during the first two weeks and the opposite effect for the next two. Reproduction traits were only significantly different in the species *E. fetida*, for which the number of juveniles decreased at higher concentrations, even showing absence of juveniles at the highest. Reproduction for *D. veneta* was not significantly affected, thus showing different sensitivity in both species. By using the filter paper contact test (OECD, 1984), the potential harmful effect by direct contact of BPA showed to be much higher than in soil (resembling natural) conditions, provoking mortality at much lower concentrations. Coelomocytes viability was not influenced, indicating that BPA may not be affecting their immune system. For *Eisenia fetida*, primer pairs were designed in order to evaluate gene expression changes between concentrations in both soil cultures and contact tests. Studied regions were involved in endocrine pathways (EcR, MAPR, AdipoR), detoxification (GST, Mt), stress (HSC70, SOD), epigenetics (DNMT1, DNMT3b, Piwi2) and genotoxicity (PARP1). Research funded by the Spanish Government. Projects: CTM2015-64913-R, CGL2013-42908-P and Postdoctoral Fellowship FPDI-2013-16407.

WE020
Alteration of Phase I and Phase II genes by single exposure and binary mixtures of Octocrylene and OD-PABA on Chironomus riparius
A.M. González, UNED / Mathematical Physics and Fluids; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos
Ultraviolet filters are main components of many personal care products that are used extensively. They are organic compounds defined as emergent contaminants that are increasing their presence in the environment, mainly by recreational activities and their use in a wide variety of industrial products. They have been associated with endocrine disruption activity in vertebrates but their effects in invertebrates are still poorly understood, especially in mixtures. *Chironomus riparius* is a diptera with aquatic larvae frequently used in toxicity tests. Previously we have analyzed the effects of octocrylene (OC) and 2-ethylhexyl 4-dimethylaminobenzoate (OD-PABA) showing that fourth instar larvae are affected by OD-PABA increasing the mRNA levels of ecdysone receptor and Hsp70 genes while OC do not show any effect in the conditions tested. In this study we have analyzed the response of fourth instar larvae to single exposure and to binary mixtures of these genes. Eight genes related with detoxification mechanisms were selected and mRNA levels were analyzed by retrotranscription and Real-Time PCR. Phase I reactions involve frequently cytochrome P450 enzymes while Phase II reactions can be carried out by glutathione-S-transferases (GST). Four cytochrome P450 genes (cyp4d2, cyp6a13, cyp9f2b, cyp12b1) and four GST genes (GST delta 6, GST epsilon 2, GST omega 1, GST theta 3) were identified from a transcriptome project and used to perform the analysis. Results showed that these genes had differential expression suggesting that detoxification mechanisms could be a source for biomarkers since several families of cytochrome P450 and GSTs have been identified in different organisms. On the other hand, single exposures and binary mixture of these UV-filters showed differences suggesting an interaction of OD-PABA and OC. Further analysis is required to understand the mode of action of UV-filters and the interactions that they can show in mixtures. This work has been funded by the Ministerio de Economía y Competitividad, CICYT (SPAIN), CTM2015- 64913-R.

WE021
Effects of endocrine disrupting chemicals (EDCs) to earthworm Eisenia andrei
R. Cui, Konkuk University / Department of Enviromental Sciences; J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Enviromental Sciences
This study evaluated the effects of bisphenol A (BPA) and bis(2-ethylhexyl)phthalate (DEHP) to earthworm. Test earthworm was Eisenia andrei that was exposed at 10 g of control or exposed soils for 7 days. The adverse effects were measured at individual and cell levels. At individual level, the mortality, abnormality (fragment, thinning, swelling, bleeding, and mucous) and burrowing rate were measured. At cellular level, the coelomocytes cells were extracted from earthworms to assess esterase activity and oxidative stress. As results, there were no significant effects for DEHP at individual levels, but esterase activities were significantly decreased at 200 mg/kg and 20 000 mg/kg dry soil at cellular levels. For BPA, adverse effects at individual and cellular levels were significant at 300 mg/kg dry soil. There were no significant effects of oxidative stress for both DEHP and BPA. These results can be used for environment assessment of BPA and DEHP in soils. *This study was funded by the Korea Ministry of Environment (MOE) as the Environmental Health Action Program (RE201603033), and Graduate School of Specialization for managing information of chemical risk.*

WE023
Effects of PFAS on the thyroid function of Perch (Perca fluviatilis)
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The presence of per- and polyfluorinated alkylated substances (PFAS) in the aquatic environment can have severe effects on the health of aquatic organisms. PFASs are man-made, highly persistent and surface active compounds which are

able to bio-accumulate through food webs and have been shown to possess toxic properties. Adverse effects include disruptions of the endocrine system, developmental and reproductive toxicity as well as effects on immune function. In 2013, elevated levels of PFASs were detected in one of the waterworks in the municipality Ronneby in southern Sweden. The source of this contamination was firefighting foam used by a nearby airfield since the 1980s. The levels of perfluorooctanesulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS) were very high in serum from the human population in Ronneby and elevated levels of PFOS, Perfluorooctanoic acid (PFOA) and PFHxS were also found in water and fish sampled from lakes the area. The current study aims to determine effects of the elevated PFASs levels on fish living in the contaminated lake Sänksjön in Ronneby. The project is being performed in parallel with epidemiological studies of the human population living in Ronneby. Since the area is relatively pristine with few sources of pollution other than the firefighting foam, this site provides us with a unique opportunity to study effects of the PFASs on fish in a natural environment. European perch (*Perca fluviatilis*) was selected as a model since our research group uses it for biomonitoring and since we have recently sequenced the transcriptome of *P. fluviatilis*. During autumn 2016, female perch were sampled from the contaminated lake Sänksjön downstream of the airfield in Ronneby as well as from a reference lake. Our results indicate that the females sampled from the contaminated site have a lower condition factor and a larger gonad size than those from the reference site. The initial focus will be to determine the impact of the PFASs exposure on thyroid related parameters in the perch. Thyroid hormones T3 and T4 will be quantified in the plasma and quantitative PCR (qPCR) will be used to measure effects on expression of thyroid related genes including thyroid-stimulating hormone (TSH), thyroid receptor α/β and deiodinases. Furthermore, inflammatory responses in the exposed fish will be examined. In addition, we will also analyze the same parameters in perch sampled from a lake in northern Sweden contaminated with PCB.

WE024

COMMERCIAL FISH FEED CAN CONTAIN STEROLS THAT ARE ENDOCRINE DISRUPTORS

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The quality of feed used to feed fish in laboratory experiments to evaluate the pollutant effects on the physiology of these organisms is an essential factor for the success of the studies. The presence of substances that can alter the metabolism of the fish can completely compromise the results obtained in the experiments. The objective of this study was to evaluate the composition of several commercial fish feed traded internationally, in particular the presence of sterols. The fish feed under study were extracted with two different solvents - methanol and methyl tert-butylether, and the extracts obtained were evaporated, silanized with MTBSA and analyzed by GC/TOF-MS. For the analyzes a Zebron ZB-5MSi column was used, helium gas, temperature programming of 90 °C for 1 min, from 90 to 290 °C, 15 °C /min was used. The results obtained revealed the presence of sterols such as β-sitosterol, stigmasterol and cholesterol in concentrations in the ppm range. These sterols are abundant in raw materials such as soybeans, which have been used extensively to produce fish feed. The effects of β-sitosterol and stigmasterol described in the literature are quite controversial. Particularly in fish, several studies have shown estrogenic effects. Therefore, the use of such feeds may significantly compromise the results obtained in assessing the physiological effects of xenobiotics, particularly those that are endocrine disrupters.

WE025

TOXICOLOGICAL EVALUATION OF ABIETIC ACID IN TILAPIA (Oreochromis niloticus)

C.H. Soares, Universidade Federal de Santa Catarina / Biochemistry Department; j.O. Querino, Universidade Federal de Santa Catarina / Biochemistry; I. Baptista, Universidade Federal de Santa Catarina

Acid resins, such as abietic acid, are terpenes produced by coniferous trees such as Pinus. Such compounds present great resistance to biological and chemical degradation that associated to its hydrophobic nature can potentiate the toxic effects through bioaccumulation effect. The objective of this work was to evaluate the toxic effects of different concentrations of abietic acid (10 mg / L and 50 mg / L) in tilapias (*Oreochromis niloticus*). The fish bioassays (10 individuals per tank, per concentration and a control group) were performed at 25 °C, light/dark period of 12/12h, with water renewal every 48h for 15 days. After that period, blood samples were collected in heparinized syringes to perform biochemical analyzes - plasma concentration of cholesterol, glucose, triglycerides, estradiol and testosterone, enzymatic activity of ALT and AST, as well as hematocrit and hemoglobin concentration. Then the fish were sacrificed by cerebral commotion for collection of liver, gonads and gills for histological analysis. The results showed that the fish exposed to both concentrations of abietic acid presented reduction of cholesterol, triglycerides and glucose, significant increase of estradiol and unchanged concentration of testosterone in relation to the control group. The hemoglobin concentration was not altered and the hematocrit showed a slight elevation. Significant morphological changes of blood cells, liver, gonads and gills were observed at the two concentrations of abietic acid tested, absent in the controls. The results demonstrated that abietic acid was able to promote serious physiological and biochemical changes in the studied fish

WE026

Effects of Mercury and Pesticides on Thyroid function in juvenile fish

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Numerous environmental stressors exert acute or chronic effects on the fish thyroid cascade. Such effects could be mediated via thyroidal alterations, imbalance of plasma T4 and T3 levels or damage to the structure of the thyroidal tissues (thyroid hypertrophy, hyperplasia). The thyroidal system is intricately linked with other endocrine systems in vertebrates including the control of reproduction. Disruption of fish thyroid function by environmental stressors has the potential to result in deleterious effects including the inhibition of sperm production, reduction in egg production, gonad development, ovarian growth, swimming activity, fertilisation and increase in larval mortality. Thyroid hormones play a major role in the development and growth of fish, particularly during their early life stages, thus, thyroid disruption by environmental stressors could inhibit the growth of fish larvae and juveniles in wild fish and cultured species, limit fish seed production and result in a decline in wild fisheries. This study evaluated the effects of two common persistent environmental toxicants: the toxic trace metal mercury and DDE (the derivative of the organochlorine pesticide DDT) on the thyroid hormone levels in juvenile Australian Black Bream *Acanthopagrus butcheri* after exposure for 2 and 4 weeks, followed by 2 weeks depuration. Exposure to both toxicants resulted in significantly depressed T3 plasma concentrations & increased plasma T4 concentrations indicating an inhibition of the 5'-monodeiodinase enzyme in juvenile black bream. Elevated T4 plasma concentrations also indicated a failure in the negative feedback response of the hypothalamus-pituitary-thyroid axis. Although the T4 & T3 plasma concentrations did not return to control concentrations during the depuration period, the hormone ratios in the p,p'-DDE exposures approached those of the control group, indicating no permanent physiological changes at the toxicant concentrations used in experiments. The study also reviewed comparative studies on the effects of mercury and other pesticides on thyroid hormones and development of fish species worldwide.

WE027

Floodhydrotox - an interdisciplinary approach to assess the endocrine-disrupting potential of sediments during flood events

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During flood events, pollutants such as polychlorinated biphenyls, (PCBs), polycyclic aromatic hydrocarbons (PAHs), dioxins and potentially also endocrine-disrupting chemicals (EDCs) can be remobilized from re-suspended sediments, leading to elevated concentrations that may be toxic to aquatic wildlife. An interdisciplinary project named Floodsearch was conducted at RWTH Aachen University assessing the risks associated with the remobilization of particulate-bound contaminants to fish. PCBs and PAHs remobilized from the sediment were identified to negatively impact fish in a laboratory study under realistic exposure conditions using an annular flume. To date, many studies have estimated the impact of remobilized PCBs, PAHs and dioxins from the sediment to fish. However, to the best of our knowledge, little is known about the ecotoxicological relevance of sediment-borne EDCs. The main objective of the follow-up project, Floodhydrotox, is to estimate the effects of EDCs remobilized from sediments during flood events on fish, i.e. rainbow trout (*Oncorhynchus mykiss*). Furthermore, we aim to simulate a multiple stressor situation which may occur during flood events, in which not only the sediment is a source of EDCs but overflow from sewage treatment plants. Floodhydrotox is designed as a stepwise approach. Natural sediments along the River Luppe in the area of Leipzig, which was already reported to be a hotspot for EDCs in sediment by Buchinger et al. 2013, are characterized for potential EDCs using non-target analysis and effect directed analysis (EDA) in combination with the yeast estrogen screen (YES). Estrogen equivalent concentrations (EEQs) already determined in sediments from five sites from the River Luppe using the YES ranged from 15,082 up to 80,377 ng/kg dry weight, which exceeds maximum sediment EEQs reported for other sediments in existing literature by a factor of ten. Secondly, the bioavailability of endocrine disrupting chemicals from these sediments will be estimated by passive sampling. Finaly, rainbow trout will be exposed to this sediment and a number of typical biomarkers of exposure to estrogenic chemicals, e.g. plasma vitellogenin and steroid hormones, will be assessed. This project is among the first investigating the impact of sediment-borne EDCs on fish, which is of great importance in context of the increasing frequency of flood events and the observed decline of many fish populations globally.

WE029
Data Integration and Gene Network Analysis for Identifying EDCs in Personal Care Products Associated with Breast Cancer
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Endocrine disrupting chemicals (EDCs) impede the actions of hormones and may lead to a variety of adverse health effects (e.g. infertility, cancerous tumors and birth defects). According to exposure monitoring data, EDCs are detected in human serum, breast milk, urine samples as well as even in placenta. Personal care products (PCPs) are widely used globally and contain diverse chemical additives used for preservatives, fragrances, disinfectant and improving their function. From animal and clinical studies, some chemical ingredients in PCPs are revealed to induce estrogenic effects. Several epidemiologic case studies found that exposure to estrogen-mimicking EDCs from consumer products could change serum hormone concentration. Long term exposure to exogenous hormones could also cause detrimental biological effects despite very low concentration. Estrogen plays a role in the etiology of a breast cancer. Breast cancer is one of the most common cancer among female. Although synthetic substances have possibility to cause estrogenic activities, knowledge on what chemical components in PCPs can relate to breast cancer development is still lack. The objective of this study was to identify linkage between potential EDCs in PCPs and breast cancer by gene network analysis and data integration using bioinformatics tools. Through bioinformatics approaches, we can integrate dispersed individual data and derive unknown common truth. From this study, we could find top interacting genes and potential EDCs which related to cause breast cancer and by combining epidemiologic and genomic data, it suggest molecular linking between exposure to EDCs and development of breast cancer.

Behavioural ecotoxicology: Unravelling behavioural responses to aid environmental and regulatory toxicology (P)

WE030
The use of high-throughput image analysis to cost effectively assess ecological relevant behavioural changes in Daphnia magna exposed to ng/l of psychiatric drugs.
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One of the major challenges that faces today regulatory risk assessment like REACH is to speed up the way of assessing threshold sublethal detrimental effects of existing and new chemical products. The recent development of different video-tracking software in neuroscience research has enables standardize and automate behavioural endpoints, promoting reproducibility and allowing for multiple endpoints to be recorded at once. Light is a common stressor for many different organisms, and many invertebrates respond to light increasing locomotion. The water flea Daphnia magna exhibits strong escape response to light, an ecological relevant response aiming to avoid fish predation. Consequently, daphnids migrate to deep dark waters during daylight and to surface waters during night. The aim of this study was to develop a high throughput photomotor assay based in determining the anxiolytic/anxiogenic behaviour observed in D. magna pre-exposed to different psychiatric drugs at environmental relevant concentrations. Experiments were conducted using EthoVision XT video tracking softwate and the DanioVision Observation Chamber (Noldus). Changes in speed, length travelled and preferred area (periphery vs center) were analysed and compared under several cycles of dark and light stimulus. Obtained results indicated that light was an efficient anxiogenic stimuli in Daphnia, increasing dramatically the speed, length travelled and the preference for peripheral areas. Low concentrations of psychiatric drugs like fluoxetine (10-1000 ng/L), which is the active component of Prozac, decreased significantly the effects of light. Results of this test with additional drugs (carbamazepine, diazepam, venlaflaxine) will also be presented. This work was funded by the Spanish Ministry of Science and Innovation (CTM2014-51985-R)

WE031
Using Danio rerio swimming behaviour as a standard high throughput technique for screening nanomaterial ecotoxicity.
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Danio rerio (zebrafish) is being developed as a model organism for applications in several fields of research forming a link between cell culture models and mammalian models. The advantage of a rapid reproduction cycle permits zebrafish bioassays to be studied all the way from embryo level up until adult each with different end points. The tests are also relatively rapid, inexpensive and straightforward to perform. The close homology between the human genome and the zebrafish genome make it a model candidate for behavioural and sub cellular toxicity testing, the genetic parallels include physiological and anatomical similarities between the endothelial cells, blood brain barrier, social interactions and immunogenic responses. Zebrafish have a large range of thermal tolerance range and have been reported to be able to adapt from 6.2 to 41.7°C. Adult *D. rerio* were housed in a Tecniplast Zebrafish housing system at 26°C were transported to a behavioral room kept at 25°C and individually placed into 1.1L Tecniplast tanks in front of a frontal camera setup. Fish were exposed to 2mg/L and 10 mg/L of cadmium tellurium quantum dots with three functional groups (COOH, PEG and NH_3) following standard OECD protocols and video recordings were taken for the duration of the 96 hour test. Video parameters were set to record at 25 frame rates per second using adult fish digitally marked at their center point. Seven fish were tracked per exposure concentration and compared to a control. Different arenas were setup, an upper and lower section was marked on each arena to determine where fish spent most of their time, arenas were scaled according to the dimensions of the tank (upper and lower). Fish were left undisturbed for the duration of the recordings. Video recordings were interpreted by physically viewing them and interpolating any missing data points by placing the marker on the animal in the coordinates it appeared in the arena as well as reassigning any incorrect data points where the software had lost sight of the organism. Data was imported to GraphPad using twenty four hour intervals for a period of ninety six hours and statistics were determined using One Way ANOVA's to compare fish over time and temperature change with Tukey's post hoc tests, significance was seen where $p < 0.05$.

WE032
Behavioural effects of pesticides and pharmaceuticals on chironomid larvae (Diamesa zernyi) from a glacier-fed stream
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Traditional standardized toxicity tests, are based on the measure of well-defined endpoints; however, they do not provide information neither on the effect at sub-lethal concentrations (e.g. alteration of organism's behaviour), nor about indirect effects (prey predator interactions). In the last years, studies on behavioural changes due to chemical exposure are constantly increasing. One of the most important issues in performing behavioural studies is the identification of a specific endpoint. For the invertebrates, alterations in movement are the most common endpoints utilized to determine behavioural effects after chemical exposures. Movement is a requirement for some activities such as food search, reproduction and predator avoidance, etc. changes in the movement ability may affect the fitness of an organism and, in turn, lead some effects at population, community and ecosystem levels. The recent advances in quantification method for behavioural patterns, such as computerized video-tracking procedures, make them valuable biomarkers useful as early warning indicators of chemical stresses. In this context, the aim of this study was to evaluate the movement changes of the chironomid larvae (*Diamesa zernyi*) after the exposure (24, 48, 72 hrs) to several pesticides (chlorpyrifos, S-metholaclor, boscalid, captan) and pharmaceuticals (ibuprofen, furosemide and thrimethoprim). All chemicals were tested at sub-lethal concentrations. Some of these have been found in trace in the stream where larvae were collected, a glacier-fed stream in the Italian Alps. Typically these remote habitats are colonized, almost exclusively, by chironomids belonging to the cold stenothermal and stenotopic genus *Diamesa*. The movement of the larvae was investigated using the ImageJ Plugin wrMTrck video-tracking software. This analysis highlighted pollutant- and time- dependent effects on the larval behaviour in terms of movement frequency. For example, some pollutants seem to inhibit or affect the mobility and type of movement of the larvae (e.g. S-metolachlor and trimpetoprim after 72hrs of treatment) while others seem to accelerate or increase the mobility of the larva (e.g.boscalid after 48 and 72hrs of treatment). These preliminary results are very promising in order to better understand the pollutant effects on freshwater organisms as influence on individual behaviour and indirectly on the health and fitness of higher hierarchical levels of biological organization.

WE034
Determining the effects of antidepressants on multiple behaviours in crustaceans at environmentally relevant concentrations
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Behavioural assays have been gaining recognition as a viable endpoint in ecotoxicology as they provide a link between biochemical and ecological effects of environmental contaminants. Psychotropic drugs are designed to modulate behaviours in humans, and preclinical studies have demonstrated that these compounds can also alter behaviours in aquatic vertebrates. The effects of behavioural modulating drugs have been tested from a pharmacological discipline using anxiety-like behaviours including thigmotaxis and scototaxis on mice and zebrafish, using well-defined behavioural assays. These pharmacological methods have been translated to ecotoxicological studies on vertebrates but comparatively few have been done on invertebrate species. Furthermore, differences in experimental design has resulted in difficulties in differentiating between closely related endpoints. For example, negative phototaxis can be interpreted as scototaxis and positive phototaxis can be interpreted as anxiety-like behaviours. This ongoing study aims to translate these techniques to model crustaceans for the purpose of high-throughput assessment of environmental risk using the antidepressant fluoxetine as a model compound. Specimens of the amphipod, *Echinogammarus marinus* were exposed to environmentally relevant concentrations of fluoxetine

from 0.001 to 1 µg/L during 1 day, 1 week, and 2 week exposures. In choice experiments *E. marinus* showed an increase in sensitivity to the light and increased swimming speed with concentrations as low as 1ng/L and as soon as 1 day compared to controls (p=< 0.05). Choice experiments are currently being translated to a freshwater amphipod *Gammarus pulex* and optimised for *Daphnia* and *Artemia* for high-throughput screening. Results indicate that fluoxetine has significant effects on velocity and phototactic behaviour at environmentally relevant concentrations. No significant effects were observed on thigmotaxis. These results may have implications for future study design of these types of experiments and aid the development of high-throughput analysis on common laboratory invertebrate species.

WE035

Habitat preferences of linnets (Linaria cannabina) in vineyards

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A large part of vineyards in Europe show no or very little ground vegetation, due to chemical and non-chemical weed control. But management techniques have started to change in the last years resulting in a reduction in herbicide applications and in an increase in ground vegetation growth and cover. This in turn, could lead to a higher attractiveness for wildlife, especially for foraging birds. However, what type of ground vegetation (e.g. structure and composition) is important for birds remains quite unknown. Here, we use habitat selection models to fill this gap and to show habitat preferences of foraging linnets (*Linaria cannabina*) in relation to the ground vegetation of commercially used vineyards in different regions in Switzerland and Germany. Habitat preferences were measured on two different spatial scales. Firstly, on a landscape scale we monitored birds over the yearly cycle in Switzerland only. Secondly on a field scale we compared vineyard characteristics of presence points (chosen by linnets) with pseudo-absence points (not chosen by linnets). Our results highlight the importance of ground vegetation in vineyards, which should allow designing precise season-specific management recommendations to promote linnets specifically and avian biodiversity in general.

WE036

Behavioral responses to urban pollutants in a key bioturbator species, the oligochaeta Limnodrilus hoffmeisteri

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In urban environments, many contaminants were accumulated on sealed surfaces (roads, parks, walkways, industrialized areas...). Rainfall events drained these pollutants, which are finally accumulated in the sediment compartment of stormwater infiltration basins. These structures were designed to detoxify runoff water that reached out groundwaters. Thus, some pollutants (heavy metals, PAH) could be highly concentrated in infiltration basin sediments. Only a few aquatic species are able to maintain populations in such biotopes, which implies specific physiological and behavioral adaptations to cope with these harsh conditions. The aquatic worm Limnodrilus hoffmeisteri is abundant in this extreme environment and plays an essential functional role. Indeed, this key species burrows galleries (bioturbation activity) in the sediment, favoring biogeochemical processes (organic matter mineralization, nutrient recycling) and microbial activities (bacterial colonization of the galleries' walls). But their physiology and behavior could be impacted by high pollutant concentrations, probably impacting the infiltration basin functioning and therefore the groundwater quality. To evaluate the impact of urban pollution on the functional role of L. hoffmeisteri, we studied the change in bioturbation activity and galleries network (density, vertical distribution, morphology) in worms incubated in four sediments (from three infiltration basins, more or less polluted, or in a low polluted stream sediment) during 30 days, using X-ray micro-tomography (with a 13µm resolution). We hypothesized that sublethal concentrations of urban pollutants affects worms burrowing capacities and behavior. We studied the abundance and the distribution of galleries (depending on depth) and we observed changes in the number and the diameter of biogenic structures (galleries distribution along the column of sediments depending on the pollution level). We might explain these results by an escaping behavior displayed by worms to find an unpolluted area. We also could suggest that the change in burrowing activity shown by worms was influenced by the amount of organic matter in the sediments. We might think they had a preferential feeding zone in the upper layer of polluted sediments where they could select the less polluted fine particles for their energetic requirement to cope with pollutants.

WE037

Behavioral tests with terrestrial crustacean Porcellio scaber as fast screening tool for agricultural application - an example of carbonized materials

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Agricultural soils amended with carbonized materials (chars) are subjected to changes from their natural state with potential impact on soil fauna communities and soil ecological processes such as nutrient cycling. In this study, we investigated how the addition of different carbonized materials (olive mill pomace biochar - BC_OMP and urban greens pruning residues hydrochar - HC_UGPR) affect the behavior of terrestrial isopods *Porcellio scaber*. These invertebrates are an important part of soil fauna, having major roles in decomposition of organic material and mixing of the soil. However, up to date no study has been done on the potential effects of chars on isopods. Isopods were exposed to soil amended with chars at different agriculturally relevant application doses. Selection behavior of isopods towards chars, in single and multi-soil selection tests, was first investigated in short term exposure (48 h). Second, we exposed the isopods to chars amended soils for a period of 14 days and followed their alterations in food consumption and body mass. Finally, we conducted food selection tests offering chars as pure material, sterile and nonsterile, to exclude microorganisms inhabiting chars as the reason for isopod selection behavior towards chars. We showed that isopods are effectively able to choose between chars amended and un-amended soil. They demonstrated clear preferences for BC_OMP, but very obviously avoided the HC_UGPR char. No differences in this preference/avoidance behavior was detected when the chars were sterile, meaning that isopods were able to choose between different chemical compositions of chars. Long-term exposure showed that avoidance to a certain char may have adverse effects on isopods. Those specimens that were exposed to HC_UGPR char consumed less food and had lower body mass, while quite the opposite was found for BC_OMP. We suggest that behavioral tests with *Porcellio scaber* could be used as fast, responsive, reliable and economically feasible screening method for the safety of chars for soil environment.

WE038

Running hot and cold: Integrating behavioural assays and thermal imaging to assess the effects of fluoxetine in birds

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Whether or not animals respond appropriately to novel and/or stressful stimuli will directly impact upon survival. The antidepressant fluoxetine (Prozac) has been designed to modulate human behaviour at low doses, specifically anxiety-related behaviours. Anxiety is predicted to be reflected in behaviours and physiological markers linked to stress responses. Here, we tested whether starlings *Sturnus vulgaris* exposed to fluoxetine at concentrations reflecting exposure via feeding on invertebrates from wastewater treatment plants, showed alterations to their behaviour or physiology. Fluoxetine-treated birds became more lethargic and less active than controls relative to their pre-trial activity levels. They also became less exploratory in a novel environment and less neophobic towards novel objects than controls. Non-invasive measures of stress, specifically corticosterone metabolites in faeces and skin temperature, as measured by thermal imaging, were used to investigate physiological mechanisms underpinning the observed behavioural changes. To conclude, less anxious and less active birds are predicted to take inappropriate risks or fail to evade predators, resulting in lower survival but this remains to be tested in the wild. Thermal imaging to record changes in skin temperature due to sympathetically-mediated vasoconstriction, potentially provides a relatively quick and easy method of assessing contaminant-induced stress in homeotherms. Moreover, key behaviours, for which there are established measurement protocols from the behavioural ecology literature, can provide sensitive and environmentally relevant endpoints for ecotoxicological studies.

WE039

Behavioral and Physiological Responses of the Cladoceran Daphnia magna after Exposure to Neuroactive Pharmaceuticals

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Determining adverse effects of human pharmaceuticals to aquatic biota is becoming increasingly relevant. However, relatively little is known about the bioavailability and sublethal effects of many neuroactive pharmaceuticals to freshwater invertebrates. In this study, we investigated the adverse effects of neuroactive pharmaceuticals on swimming behavior, filtering activity and enzyme activity of the freshwater Cladoceran *Daphnia magna*. The investigated pharmaceuticals included diphenhydramine, propranolol, fluoxetine, and citalopram. Changes in feeding of juvenile and adult *D. magna* were determined by measuring filtering and uptake of live food items including algae and bacteria. Hydrolytic enzyme activity in *D. magna* was measured by targeting chitinase and chitobiase. These enzymes are involved in exoskeleton degradation and recycling during moulting. Changes in swimming behavior of *D. magna* was quantified by video tracking of single organisms followed by image analyses. Sublethal concentrations of the pharmaceuticals resulted in changes in behavior patterns including altered orientation of movement, swimming distance, and active swimming time. Responses measured as changes in filtering activity and swimming behavior were nonmonotonic with significant differences in time, and in responses measured at low concentrations (ng/L to µg/L) compared to elevated concentrations (µg/L to mg/L). This challenges currents models for evaluating sublethal effects of pharmaceuticals to non-target organisms.

WE040
Chlorpyrifos effects at different levels of ecological hierarchy: link between changes in biomarkers (sub-organism level) and behavioral changes in Daphnia magna (organism level)
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The consequences of the exposure to xenobiotics in natural ecosystems are still largely unknown and this stands in stark contrast to the protection goals of Environmental Risk Assessment (ERA), which include the protection of populations and communities. This is particularly true for the so-called Emerging Contaminants (ECs), whose adverse effects towards non-target organisms have been only recently highlighted, e.g. effects of drugs of abuse, pharmaceuticals and personal care products. Increasing laboratory evidences showed that the exposure to 'environmentally relevant' concentrations of different ECs may induce several adverse effects to organism, including changes in behavior or physiology, which could affect fitness, and consequently population dynamics. However, until now, there is a dearth of information on the linkages among the different levels of bio/ecological organizations. In this context, this project was aimed at investigating the effects at different levels of the ecological hierarchy of one of the most widely used organophosphate pesticide such as the chlorpyrifos (CPF), the active ingredient in a range of popular broad-spectrum insecticide formulations, on the cladoceran *Daphnia magna*. To reach this goal, *D. magna* specimens were exposed for 96 hours to two CPF sub-lethal concentrations (50 ng/L and 250 ng/L), similar to current levels found in freshwater ecosystems worldwide. Effects at sub-organism level were investigated by the application of an appropriate suite of biomarkers. Furthermore, the effects at organism level were assessed evaluating the behavioral changes in swimming activity of cladocerans by using a video-tracking analysis approach. Both the CPF tested concentrations modulated antioxidant responses (SOD, CAT and GPx activity) in treated specimens with respect to control. In addition, a significant decrease in swimming velocity and distance moved was noted, whereas inactive time increased, in treated *D. magna* specimens compared to control. Our preliminary findings suggest that sub-lethal effects at sub-organism level can lead to behavioral changes in the organism and serve as early warning signal to foresee effects at higher levels of the ecological hierarchy.

WE041
Assessing the Ecotoxicological Potential of Carbamazepine - Evaluation of a Behavioural Parameter in the Embryogenesis of Zebrafish
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The pollution of the aquatic environment is currently characterised by a great number of contaminants, especially by mixtures of micro-pollutants. The ecotoxicological implications of this burden is not yet fully assessable. The anticonvulsant carbamazepine was analysed using a newly developed test. The change of frequency of spontaneous tail movements occurring during embryogenesis of Zebrafish (*Danio rerio*) was utilised as a sensitive endpoint to assess the neurotoxic potentials of substances. The method was validated using a positive control. After fertilisation, Zebrafish embryos were transferred to 24-microwell-plates and exposed to carbamazepine. The embryos were incubated at 28,5 ± 0,5°C. Effects on behaviour were examined after 24 hours of exposure by videotaping (30 seconds) each well and quantifying spontaneous locomotion in comparison to a negative control. Carbamazepine exposure showed decreasing, concentration-dependent effects on embryonic locomotion. The determined data was compared to acute and chronic ecotoxicity data found in literature. The results of the behavioural based assay displayed a considerable increase of sensitivity compared to results from the Fish Embryo Acute Toxicity Test and the chronic Fish Early-Life Stage Toxicity Test. This work illustrates the sensitivity and specificity of the established endpoint within the developed test method which makes it possible to reliably assess adverse, neurotoxic effects of compounds on the model organism *Danio rerio*. This can be a substantial contribution to an effect-based risk assessment of contaminants in aquatic ecosystems.

WE042
Behavioral responses of marine vertebrate (Solea senegalensis) early life stages after exposure to Carbendazim, 4MBC and UV-B radiation
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Senegalese sole (*Solea senegalensis* Kaup, 1858) is a promising marine vertebrate for ecotoxicological studies. This flatfish inhabits coastal areas during larval stages, completing a metamorphosis at the first month of life. Eggs for laboratory testing can be obtained from aquaculture facilities in South Europe (Portugal, Spain, France and Italy) throughout the year. Sublethal responses to contaminants and manipulation of environmental factors can provide important information on how aquatic species cope with global change in environment. Behavioral endpoints are sensitive and non-invasive tools for the evaluation of contaminants and are increasingly being used in ecotoxicology. Computer and video automation have been improving the analysis of behavior turning it more reproducible, reliable and less time-consuming. Therefore, the aim of this work was to understand the behavioral response of *Solea senegalensis* to different classes of stressors (a fungicide, an UV filter and UV-B radiation). Carbendazim is a fungicide widely used in agriculture and their adsorption to sediments is expected in aquatic ecosystems. This benzimidazole is a suspected carcinogenic compound and might cause hormone disrupting effects in vertebrates. 4-Methylbenzylidene camphor (4MBC) is an UV filter broadly used in sunscreens and other personal care products and is also referred to cause endocrine effects. UV radiation penetrates the ocean's surface and may cause negative effects to marine species. Decreasing levels of stratospheric ozone have been reported in response to increase of artificial ozone-destructing compounds, resulting in increasing levels of ultra-violet radiation on surface of Earth. At the beginning of the metamorphosis (13 days after hatching), fish were exposed during 48 hours to a range of sublethal concentrations of Carbendazim and 4MBC and to UV-B radiation. After the completion of the pelagic-to-benthic metamorphosis, behavioral response to light stimulus was tested using a Zebrabox (Viewpoint, Fr). This device radiate specific light, uses an infrared camera and records swimming parameters of exposed fish. In each treatment fish were exposed to two light-dark cycles of 15 minutes and duration of swimming, distance of swimming and number of movements were evaluated. Behavioral responses to the stressors will be discussed in regard to their impact in competition, prey-host interactions and feeding behavior and conclude about the relevance of behavior for risk assessment purposes.

WE043
The critical window of exposure of mixture toxicity of benzene, toluene and formaldehyde in Danio rerio: A systems toxicology approach with behavioral analysis
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In this study we aimed to investigate the potential hazard of mixture toxicity of volatile organic compounds (VOCs) (such as, benzene, toluene and formaldehyde) as well as their individual toxic potentialities, we performed a systematic analysis with zebrafish (*Danio rerio*) by using comparative systems toxicology approach. At first we conducted pathway analysis based on microarrays of the individual chemicals followed by experimental validation with standard toxicity endpoints (such as, mortality, embryo toxicity etc.) as well as behavioral analysis. To explore the molecular mechanisms, we carried out global DNA methylation and selected respective gene expression analysis (such as dnmts, cat, vtg1, cyp1a, gstp, sod2, erb, serpinc1 etc.). In addition, we carried out early life exposure modulate later life outcome of these VOCs and their mixture by demonstrating that the acute exposure (24h) in very early life stages (at 4 hpf) of zebrafish led to more changes in its behavior aberration than the same exposure in 28 hpf stages compared with the control. All behavior measurement was performed at 10dpf and it revealed that early exposure shows decrease in acceleration, speed, and turning rate whereas increase in stop duration and meander. Taken together, in the present study we exhibit a paradigm of critical window of exposure, i.e., how the early life exposure induce molecular as well as epigenetic changes which came up with phenotypical (aberrant behavior) alterations in adults and we believe our results could eventually could be utilized for better risk assessment of the respective chemicals. Keyword: Zebrafish, Behavior, Mixture toxicity, Benzene, Toluene, Formaldehyde

WE044
Application of Automated Biomonitoring in India
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Indian inland water bodies are getting polluted day by day due to the continuous discharge of semi-treated and untreated effluent from various sources into these water bodies without considering the threat to aquatic life and human health. More than attaining some water quality standard values, the survival of an organism in the water bodies without stress is important. However continuous and online monitoring of this is not possible by physical and chemical analysis. The objective of present study was to apply Multispecies Freshwater Biomonitor (MFB) in various fields in. As a first application of MFB in India, usefulness of *Poecilia reticulata* (*Guppy fish*) as an indicator organism for bio-monitoring the effluent quality from a sewage treatment plant (STP) in India has been assessed. Using Multispecies Freshwater Biomonitor© (MFB) developed by LIMCO International GmbH, the behaviour patterns of *P.reticulata* in 5, 20, 50, 75 and 100% of STP effluent concentration with reference to a control (open well water) were analysed. The dilutions at which *P.reticulata* gives an early warning for the risk to their life

were identified. Meningeal blackness was observed as a non-invasive indicator of stress level in *P.reticulata.* In the study of effect of acute toxicity of three micro pollutants: acetaminophen (paracetamol), carbamazepine and ibuprofen on *P.reticulata* and *D.rerio*; both the species were exposed to different concentrations of the micro pollutants for 24 hours. Their behaviour in the control water and various concentrations were observed using MFB. The study showed that there is a direct impact on fish species with increase in concentration of toxicant. Acetaminophen was found to be more toxic compared to other micro pollutants tested. In the study of effect of salinity on two different fish species namely *P.reticulata* and *D.rerio* by observing their behaviour in the control water and various concentrations using MFB *P.reticulata* tolerated relatively high concentration of salinity comparing to *D. rerio*. Also *P.reticulata* shows avoidance behaviour (increased movement activity) at low concentrations of salinity whereas in *D. rerio* the main impact of salinity was movement activity reduction at all concentrations. The findings from this study are favourable for the propagation of automated biomonitoring in India and for real time monitoring of the water quality degradation due to sewage pollution.

WE045

Acute toxicity and recovery of a Cu-pulse: G. fossarum vs. N. casparyi and P. slavus

A. Gerhardt, N. Leubner, C. Grimm, A. Petri, LimCo International GmbH

Copper has been used in many different applications worldwide and still is a significant threat for life even though at low concentrations essential for cellular functions. Copper is being used for drinking water pipes hence a threat for human health. Searching for sensitive aquatic indicator species for continuous groundwater and drinking water monitoring we compared adults of two freshwater amphipods, G. fossarum and N. aquilex/casparyi as well as the isopod P. slavus in their sensitivity towards acute Cu-pulses (24 h) and their resilience after 7 d of recovery. Seven concentration levels between 5 and 720 ug/l were tested using the Multispecies Freshwater Biomonitor (MFB) to record quantitatively and continuously the individual survival and behaviour (locomotion). Locomotion decreased in a concentration-dependent manner in both N. casparyi and G. fossarum, the behavioural effects remained after recovery. P. slavus showed a trend to increased activity at higher Cu-levels. Regarding capture at field sites, culture and maintenance in the laboratory and Cu-sensitivity G. fossarum remains the most appropriate test species. The study has been co-financed by the BMBF-project GroundCare.

WE046

Altered behavior and skewed sex ratio after developmental exposure of 17α-ethinylestradiol in the three-spined stickleback (Gasterosteus aculeatus)

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Endocrine disrupting chemicals (EDCs) are ubiquitous in the aquatic environment and can interfere with the hormone system through multiple ways. Many of the reported examples of endocrine disruption in wildlife are of estrogens and their mimics. 17α-ethinylestradiol, EE2, is a synthetic estrogen commonly detected in sewage effluents. Recent studies have shown persistent effects on the behavior of zebra fish and guppy developmentally exposed to estrogenic compounds. In this study we exposed the three-spined stickleback (Gasterosteus aculeatus) to 20 ng/L of the synthetic estrogen EE2 during development. Exposure started at the day of fertilization and continued on for 7 weeks. After 7 months of remediation in clean water three non-reproductive behavioral tests (novel tank, shoaling and scototaxis) were analyzed as well as sex ratio in the two groups. The fish exposed during development had significantly higher occurrence of fish with eggs and showed altered behaviors in all three of the behavioral tests. In the novel tank test developmental exposure increased number of transitions to the upper half of the aquaria as well as decreased time spent freezing. The exposed fish also had an increased number of crossings to the black compartment in the scototaxis test. In the shoaling test fish that had been exposed to EE2 during development took less time to leave the group after establishing contact and also made more transitions to the other side away from the shoal. In this study we show the effects of EE2 exposure during development in the three-spined stickleback after a long period of remediation on stress behavior and shoaling, behaviors of significance in wild fish populations and that are likely to affect fitness and on sex ratio towards feminization.

WE047

Move it! Pesticide avoidance behaviour in a predatory mite

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For a pesticide to be registered for use, the lethal and sublethal effects on non-target arthropods (NTAs) must be studied. Sublethal effects such as behavioural changes have been reported in NTAs exposed to some pesticides, with avoidance behaviour – where individuals display signs of irritation or repellence when exposed to a pesticide – being of particular interest. More research is necessary to better understand pesticide avoidance behaviour so that population consequences of such behaviour can be estimated. We aim to quantify avoidance behaviour and changes in movement behaviour in relation to pesticide exposure in the predatory mite *Typhlodromus pyri*, a model species and natural predator found in fruit orchards across the globe. Using video analysis, we conducted two types of test: the first evaluated mite movement behaviour when exposed to a range of pesticides in simple test arenas; the second investigated avoidance behaviours such as repellence through the use of a choice arena. We report movement behaviours in mites exposed to a number of pesticides at different concentrations, ranging from low to high toxicity for *T. pyri*. Particular focus will be placed on avoidance behaviour. Our results will add to existing knowledge of avoidance behaviour caused by pesticide exposure, and will also feed into an individual-based model that will predict the population-level consequences of any behavioural changes.

WE048

Toxicity of BPA: Are groundwater crustaceans N. casparyi and P. slavus more sensitive than G. fossarum?

A. Gerhardt, J. Ritzel, M. Urban, LimCo International GmbH

BPA (Bisphenol-A) is being used worldwide in plastic products and threatens the aquatic environment during degradation of plastic wastes and migration into food and drinking water from coated cans and pipes. Searching for sensitive aquatic indicator species for continuous groundwater and drinking water monitoring we compared adults of two freshwater amphipods, *Gammarus fossarum* and *Niphargopsis casparyi* as well as the isopod *Proasellus slavus* in their sensitivity towards acute Cu-pulses (24 h) and their resilience after 7 days of recovery. Moreover, chronic toxicity tests (4 weeks) were performed with sublethal concentration levels. Five concentration levels between 0.2 and 50 mg/L were tested in a static test design, where the individual animals were exposed in the test chambers of the MFB to continuously record the quantitative behavioural responses: initially in stream water, followed by 24 h of BPA-exposure. Thereafter, the animals recovered in stream water at constant temperature with aeration and food for 7 days in the dark, before they were recorded in the MFB again. The MFB records automatically survival and behavior (locomotion). Finally, at 4 sublethal concentration levels of BPA (between 0,01 and 1 mg/L) chronic effects on behavior and feeding were studied. Regarding acute toxicity (survival and locomotion) *N. casparyi* was the most sensitive species, followed by *P. slavus* and *G. fossarum*. Chronic expires (4 weeks) revealed significant effects on behavior at 0,1 mg/l *(N. casparyi*) and survival at 1 mg/l (*N. casparyi, G. fossarum*). This study was co-financed by the BMBF-project GroundCare.

WE049

THE behavioral response of three aphid species to different biochemical compounds present in four different essential oils.

M.L. Samuel, University of the Free State / Zoology and Entomology

Cereal aphids can be a serious threat to several crops around the world. Their feeding may cause direct damage to plants or result in transmission of plant viruses. Compounds of essential oils are considered to be alternative controlling means of harmful insects. The aim of this study was to examine the behavioural response of aphid species, *Rhopalosiphum padi, Sitobion avenae* and *Metopolophium dirhodum* to different biochemical compounds present in four different essential oils (Limonene, Citral, Geraniol and Ocimene) at 1% concentration. This was done to identify the most repellent and the most attractive essential oils under laboratory conditions. T-maze olfactometer was used to investigate the effects of the essential oils on the behaviour of the aphids. Aphids were introduced through a central tube to make a choice between two odours by walking into one of the arms. In order to test if the essentials oils had a significant effect compared to the control, a Wilcoxon rank test was performed. Citral oil attracted all three aphid species. Geraniol oil repelled both *S. avenae* and *M. dirhodum* and there was no significant difference for *R. padi*. Ocimene repelled also both *S.avenae* and *M. dirhodum*, but attracted *R. padi*. Limonene oil repelled *M. dirhodum,* while there was no significant repellency or attractancy observed in either *S. avenae* or *R. padi*. The results indicate that the landing behaviour of the three aphid species on host plants could be influenced by odours and that it may be possible to control aphids with attractants and repellents.

Big data analysis of monitoring data: what questions can be addressed? (P)

WE050

Metabarcoding of eDNA from Suspended Particulate Matter (SPM) for fish population monitoring

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The evaluation of fish populations and biodiversity in aquatic environments is usually performed by time- and cost-intensive studies, and often presents only a snap-shot of the actual fish population. To date, the conventional method used for this purpose is electro fishing. However, monitoring of rare species is often unsuccessful with this method. Furthermore, this method results in increased stress levels for fish and is thus also critical in respect to animal welfare reasons. Sampling of so called environmental DNA (eDNA) allows for non-invasive species determination and measurement of their DNA abundance. Cellular material carrying the genomic information of a large number of species might be present in the aquatic environment as fish continuously release bodily components like for example excrements, mucus, scales, or even eggs to the water. In this study we used metabarcoding as technique to analyse the whole species community. DNA was extracted from suspended particulate matter (SPM), which was sampled at different riverine sampling points in Germany. SPM samples were retrieved from the German Environmental Specimen Bank (ESB; Umweltprobenbank) which archives annually sampled SPM material since 2005 at temperatures below < 150°C. We assume that the material contains plenty of yet unused information on fish populations and possible temporal developments. For extraction, a method was developed which resulted in a good DNA yield and purity. We tested different PCR primer pairs, which all bind to conserved regions of the genome. These regions for example encode genes for CytB, 12S, and COI. Primer pairs described in the literature as well as newly designed primers were tested. The final approach, resulting in reliable and repeatable results on diverse SPM samples, was a nested PCR approach with newly designed PCR primers, amplifying a fragment of the COI gene. The PCR products were analyzed by Next Generation Sequencing (NGS). The results allowed the identification and discrimination of fish species. The data obtained were used for comparisons with fish monitoring data from electro fishing. With the described approach, we developed a method to reliably identify and discriminate fish species, which allows fish monitoring in a non-invasive way. By using archived SPM samples of the German ESB as starting material, this method allows for retrospective monitoring of fish populations.

WE051

Inventoring biodiversity: the strengths and limitations of DNA barcoding and DNA metabarcoding for environmental monitoring

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Proper species identification is an important prerequisite in any biodiversity monitoring program aimed at assessing ecosystem quality and health. Species identification is traditionally based on morphological characters, which is time-consuming, expensive, and requires considerable taxonomic expertise. Invertebrate taxa are particularly challenging, due to their high levels of cryptic diversity, frequent morphological stasis, lack of diagnostic characters in immature stages, and the so-called taxonomic impediment. As a result, taxonomic ranks higher than species are often used in monitoring studies, which may potentially overlook species-specific responses to environmental stressors. Alternatively, a small number of well-studied species are designated as indicators to infer the effects of environmental change on overall diversity. The use of DNA-based methods can overcome many of these issues and thus offer a complementary solution to explore species diversity. In particular, DNA barcoding (i.e., the use of a short genomic region, the DNA barcode, for species-level identification) has proven useful for delimiting and reliably identifying species in many taxonomically difficult groups. Likewise, implementing DNA barcoding in monitoring programs can improve species resolution and help diagnose the specific factors impacting an ecosystem. Moreover, DNA barcoding coupled with next-generation sequencing technologies (DNA metabarcoding) now allows the massive sequencing of thousands of barcodes to identify multiple species in a mixed sample. With DNA metabarcoding, biodiversity surveys can move from individual specimen identification towards whole-community analysis, which makes it a promising tool for long-term and large-scale environmental monitoring. Despite the broad benefits that both methods can bring to biodiversity inventory, continued effort is needed to standardise both laboratory and data analysis protocols, in order to produce reproducible and easily interpretable results. In this work we will review the recent technological advances in DNA barcoding and DNA metabarcoding, discuss their current streghts and limitations, and outline some potential improvements for the implementation of DNA-based species identification in routine monitoring.

WE052

The Edaphobase Nationwide Field Monitoring - an approach to determine reference values for soil organism communities of different habitat types

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The protection of soil and its natural functions (e. g. organic matter decomposition and nutrient cycling, or its function as a habitat for soil organisms) is regulated in different national and European regulation procedures. These natural soil functions are provided mainly by the complex interactions of numerous organisms belonging to different taxa and trophic levels. Government agencies frequently must assess the ecological value of different sites, in particular impacts of anthropogenic activities on soil structure and function. However, these impacts cannot yet be sufficiently evaluated, mainly due to the lack of knowledge on what constitutes "normal" at a specific site (usually, control sites are not available). In other words, detailed data on the diversity and abundance of soil animals correlated with site-specific information (e. g. habitat types or soil properties such as pH or texture) is lacking. In a first phase of the German Soil Biodiversity Datawarehouse project Edaphobase, the German Federal Ministry of Education and Research (BMBF) supported the collection of data for different groups of soil animals (e. g. Oribatida, Collembola, Lumbricidae, Enchytraeidae, Diplopoda, Nematoda). In the Nationwide Field Monitoring - subproject of the ongoing second Edaphobase phase, different habitat types (arable land, grassland, forests) in four German regions are being systematically surveyed, i. e. vegetation and soil properties determined and soil animals sampled and identified using standardized methods. These collections in combination with data from Edaphobase will provide an improved basis for the assessment of soil organism communities by identifying reference values (also called "Normal Operating Ranges" (NOR)) for different habitat types. In the future, this information will be publically accessible via the online-data-warehouse edaphobase.org.

WE053

The use of the Edaphobase data-warehouse in soil risk assessment

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The aim of the Edaphobase database-project is to collect and provide ecological and taxonomic data on soil fauna (http://portal.edaphobase.org). In the ongoing second project phase, sponsored by the German Federal Ministry of Education and Research (BMBF), one sub-project deals with the evaluation and further development of the online data-warehouse for the practical use in the context of pesticide registration and soil risk assessment. With the EU Regulation No. 1107/2009/EC, the focus of risk assessment changed from functional endpoints to structural endpoints. For this reason, higher tier test systems like terrestrial model ecosystems (TME) or field trials become more important to examine whole communities under more realistic conditions. When analyzing and interpreting data out of these higher tier studies it is most valuable for all participating parties i.e. industry, consultants and authorities, to get specific ecological information on the species that are involved and possibly affected by pesticides. Information on soil organisms can be scarcely found or are currently difficult to access. In this sub-project, the first step was to compile the target information that is needed for the ecological evaluation in risk assessment. Therefore, the Edaphobase data-warehouse was extensively tested. The second step was to hand over the information about missing tools or desired applications to the Edaphobase development team. A bundle of new tools was developed and integrated in the warehouse, which are exemplarily shown in the presentation.

WE054

Relationships between forest ecosystem conditions and ecosystem services on the national scale in Germany

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The amount and quality of different ecosystem services (ES) supplied by forests strongly depend on their ecological conditions. This study used data on the conditions of 60 clusters of forest ecosystem types referring to vegetation, chemical and biological soil characteristics and climate across Germany[1]. Furthermore, available data such as the BERN database or data from the Third German National Forest Inventory were collected for assessing and mapping forest conditions. K-Means was used to create clusters of ecosystem types, which then were linked to supply capacities of three selected ES using the ES matrix approach[2]: (1) Net primary production (NPP) of timber [t TS/ha] as provisioning ES (CICES class "Fibres and other materials from plants, algae and animals for direct use or processing"); (2) Soil organic carbon (SOC) sequestration (depth 80 cm, in [t/ha]) as regulating ES ("Global climate regulation by reduction of greenhouse gas concentrations"); and (3) Potential habitat quality and function (as cultural ES). With the ES matrix approach, the capacity of eocsystem types to provide ES was displayed on a scale ranging from 0 (no relevant capacity) to 5 (very high capacity). The matrix values can be used to map ES in complex landscapes, which simplifies large amounts of data of varying quality and quantity in order to produce easily understandable tables and maps. Results of this study showed a correlation between the conditions of forest ecosystems and the ES they are able to supply. The matrix values enable the user to compare the current conditions of a forest ecosystem with its potential ES supply. The ES matrix has become a very popular method that is suitable to communicate scientific findings to policy and decision makers. The study complies with the tiered approach for Mapping and Assessment of Ecosystems and their Services (MAES[3]) of the European Union. MAES contributes to the achievement of the EU's Biodiversity Strategy 2020, specifically Target 2 Action 5. The study presented here was funded by the German Environment Agency (UBA) and contributes to the German national MAES activities. **[1] Schröder W et al. 2015. Methodology to assess and map the potential development of forest ecosystems exposed to climate change and atmospheric nitrogen deposition: a pilot study in Germany.** ***Science of the Total Environment*** **<strong>521-522**: 108-122. [2] http://www.landscapeonline.de/103097lo201434 [3] http://biodiversity.europa.eu/maes

WE055

Optimization and Accessibility of an Ecotoxicological Threshold of Concern (ecoTTC) Database

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The Ecological Threshold for Toxicological Concern, or ecoTTC, has been proposed as a natural next step to the well-known human safety TTC concept. The ecoTTC is particularly suited for use as an early screening tool in the risk assessment process, in situations where chemical hazard data is poor, or when an appropriate QSAR is unavailable. EcoTTCs are developed using statistical distributions of Predicted No-Observed Effect Concentrations (PNECs) to reflect the breadth and depth of the ecotoxicological dataset beneath, and therefore, the diversity and quality of the underlying dataset is crucial to the future utility of the ecoTTC. A database consisting of approximately 110,000 unique ecotoxicological records, 6200 unique CAS numbers and 1900 species from three trophic groups has been created based on recent assessments of published data and international chemical management programs. Stepwise data selection strategies, query systems and curation techniques were applied to ensure a transparent, methodical process towards a final dataset, which also includes reference-sourced toxicity data associated with physical chemistry data and taxonomic information for the tested chemical. In order to make these data accessible and useful to stakeholders, the dataset was transitioned from Microsoft Excel and Access into a modern MySQL format. This allows for a database format that is relational and scalable, facilitating easy access, sharing, and integration with other datasets and tools. This dataset is accessed via a web-based query system that is integrated with PNEC calculator and probability distribution tools. The novel interface allows users to explore the data, upload additional datasets, derive threshold values based on specific criteria, and explore the potential use and application of the ecoTTC concept. This poster will present the architecture, web-interface, and associated tools.

WE056

Effect of PCBs on the virulence of Gallid herpesvirus 2 (GaHV-2) in chicken fibroblasts: a model in vitro system

C. Waugh, Norwegian University of Science & Technology; V. Jaspers, Norwegian University of Science & Technology / Biology

Herpes viruses are one of the most common viral infections in humans, domestic animals, and wildlife. Latent, non-productive, herpes virus infections occur for prolonged periods of time in many species. The appearance of clinical symptoms likely indicate that individuals have been exposed to a chronic stressor. Gallid herpesvirus (GaHV-2) induces Marek's disease, a lymphosarcoma, in chickens, with a major impact on the poultry industry. Until the 1950s, the virus infection was associated with a polyneuritis syndrome, with a low rate of mortality. Concomitant with increasing industrialization and environmental pollution in the 1960s, the acute form of the disease appeared, with significantly higher mortality rates. The complex interaction between host and GaHV-2 involves viral microRNAs (miRNAs). miRNAs usually favour virus persistence in the infected host through multiple mechanisms, such as inhibiting cell apoptosis, evading the immune response and regulating host and viral genes leading to the repression of the viral lytic cycle and promotion of latency. GaHV-2 encodes two main clusters of viral miRNAs, among which expression of miR-M4 has been shown to be the direct cause of the induction of Marek's disease. Pollutants are known to alter the expression profiles of host miRNAs, but no studies have yet looked at associations between immunomodulatory organic pollutants (e.g. polychlorinated biphenyls; PCBs) and viral miRNAs. Thus, this study aims to look at the association between PCBs and viral miRNA expression in an *in vitro* model system (GaHV-2 infection of chicken fibroblasts). Here, we will describe the effect exposure to PCBs has on: 1) virulence of GaHV-2 infection (by modulation of expression of pathogenic miRNAs) and; 2) the viral growth kinetics and replication. Chicken fibroblasts will be exposed to a PCB mix (Sigma Aldrich) for 24 hours before being infected *in vitro* with GaHV-2. Triplicate samples of cells will be harvested at 1, 3, and 6 days post infection. RNA will be isolated and relative expression levels of miRNAs will be measured by polymerase chain reactions (PCR) in comparison to the endogenous GAPDH transcripts (all PCR tests are run in triplicate). Virus growth kinetics will be performed via plaque assays to look at the ability of the virus to replicate in PCB exposed cells, as well as maximum viral titres. Results will be compared with non PCB exposed control cells. The results will be presented at the conference.

Predictive models in ecotoxicology: bridging the gap between scientific progress and regulatory applicability (P)

WE057

Investigating the application of artificial neural networks to predict the bioconcentration of xenobiotics in fish and invertebrates

T.H. Miller, Kings College London / Analytical and Environmental Sciences; L. Barron, Kings College London / Analytical and Environmental Science; N. Bury, University of Suffolk / Division of Diabetes and Nutritional Sciences; S.F. Owen, AstraZeneca / Safety Health Environment

The (pseudo)persistence of pharmaceuticals and other organic micropollutants in the environment is a cause for concern among the organisms that are exposed to them. This exposure may lead to the accumulation of these types of pollutants in individuals (bioconcentration/bioaccumulation) and also through several trophic levels (biomagnification)[1].However, it is currently unfeasible in terms of time, cost and labour to assess the thousands of compounds that may enter the environment and accumulate. Thus, a rapid and novel approach is required for reliable risk assessment in persistent, bioaccumulative and toxic assessments. Herein, artificial neural networks (ANNs) were developed and optimised to predict the bioconcentration factor (BCF) of micropollutants in fish and invertebrates. Several network architectures were explored with multilayer perceptrons demonstrating the best performance. A dataset for fish consisting of organic micropollutant BCFs (n=353) was used to train, validate and test MLP models. Stepwise and genetic algorithms were used to down-select molecular descriptors (n=180) that were generated from the simplified molecular-input line-entry system (SMILES) using Parameter Client (VCC Labs). The optimised fish model containing 14 molecular descriptors was applied to a smaller dataset of BCFs in invertebrates (n=34). The fish BCF model showed good performance with the verification R^2 of 0.8191 and the test set R^2 of 0.7021. The absolute errors of the verification were 0.38 ±0.36 logBCF units and 0.53 ±0.36 logBCF units, respectively. The invertebrate ANN model showed better performance with a verification set R^2 of 0.9932 and test set R^2 of 0.9323. The absolute errors of the verification and test sets were 0.07 ±0.08 logBCF and 0.29 ±0.27 logBCF units, respectively. A sensitivity analysis of both models showed that whilst they used the same molecular descriptors the importance of the descriptors in each model changed. Overall, the application of ANNs to predict BCFs showed good performance whilst significantly reducing the resource requirements associated with PBT assessments. Therefore, the incorporation of ANNs into a regulatory framework would be beneficial to the application of risk assessments. References. 1) Arnot, J.A., & Gobas, F.A.P.C (2006). *Environ. Rev.*, 14(4), 257-297.

WE058

MOSAIC in support of statistical analyses from standard bioassay data

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology

MOSAIC stands for "*MOdeling and StAtistical tools for ecotoxicology*". In ecotoxicology, bioassays are standardly conducted in order to measure acute or chronic effects of potentially toxic substances on reproduction, growth and/or survival of living animals. MOSAIC is a user-friendly web interface dedicated to the mathematical and statistical modelling of such standard bioassay data. Its simple use makes MOSAIC a turnkey decision-making tool for ecotoxicologists, regulators, managers, NGOs and other stakeholders. Without wasting time on extensive mathematical and statistical technicalities, any user is given advanced and innovative methods for a valuable quantitative environmental risk assessment. MOSAIC is available at http://pbil.univ-lyon1.fr/software/mosaic/. Today, MOSAIC offers three operational tools : (i) $\mathbf{MOSAIC_{SSD}}$, a tool dedicated to the species sensitivity distribution (SSD) approach aiming at defining safe levels for toxic compounds in an ecosystem through the estimation of the so-called hazardous concentration for *p*% of the species (*HCp*), even when the toxicity values are censored; (ii) $\mathbf{MOSAIC_{repro}}$, which provides users with a complete statistical analysis of bioassay reproduction data simultaneously accounting for mortality all along the bioassay. $MOSAIC_{repro}$ fits various concentration-effect models within a Bayesian framework. The model that best account for the observed variability is selected to provide ECx estimates; (iii) $\mathbf{MOSAIC_{surv}}$, which provides users with a complete statistical analysis of bioassay survival data. A log-logistic model is fitted within a Bayesian framework to provide LCx estimates. Based on a toy example, this talk will illustrate how MOSAIC can help ecotoxicologists, regulators, managers, NGOs and other stakeholders in statistically but easily analysing standard survival and reproduction bioassay data.

WE059
TK-TD modelling of Lymnaea stagnalis survival under chemical pressure
S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionnary Biology; S. Preux, University Lyon / Laboratory of Biometry and Evolutionnary Biology; V. Ducrot, Bayer CropScience AG / Environmental Safety Ecotoxicology
The General Unified Threshold model of Survival (GUTS) provides a theoretical framework for analysing stressor effects on survival over time through consistent mathematical equations based on different assumptions about the stressor quantification, the compensatory processes and the nature of the death process. The key GUTS feature is that an additional mortality due to the stressor occurs when the dose metric exceeds a certain threshold. Several GUTS formulations can be derived according to the assumption underlying the death process: (i) the threshold value varies between individuals and when exceeded, the individual dies (individual tolerance, IT); (ii) there is one common threshold for all individuals, and when exceeded, the probability to die increases with the concentration (stochastic death, SD); (iii) a unification of both previous assumptions (GUTS proper). While more comprehensive, GUTS proper requires the estimation of one additional parameter. This poster presents results on TK-TD modelling of *Lymnaea stagnalis* survival after long-term exposure to a cadmium concentration range. We analysed 22 datasets coming from two ring-tests performed between 2012 and 2015. We compared the two R packages ('morse' and 'GUTS') for their performance in providing robust estimates of all parameters of the different GUTS formulations. In addition, we quantified the between laboratory variability based on the different LC50 estimates (expressed as median values and 95% credible intervals).

WE060
Review, ring-test and guidance for TKTD modelling
R. Ashauer, University of York / Environment; T. Jager, DEBtox Research / Dept of Theoretical Biology
The additional information and insight gained through the application of toxicokinetic-toxicodynamic (TKTD) modelling can strengthen the environmental risk assessment of chemicals, such as those applied in consumer products or plant protection products (PPPs). For the endpoint survival the most suitable and powerful tool is currently the General Unified Threshold model of Survival (GUTS), which unifies almost all of the previously published TKTD models for survival. Specific models can be derived as special cases of GUTS. The simples cases comprise the complimentary models: GUTS-SD (stochastic death) and GUTS-IT (individual tolerance). GUTS has been submitted as part of the environmental risk assessment of PPPs, but it can also be used within the context of REACH and other risk assessment frameworks. In order to facilitate application of GUTS and increase trust and acceptability we need to compare and test the range of software implementations available and generate guidance on how to use GUTS in Environmental Risk Assessment of chemicals. Beyond the endpoint survival, and beyond GUTS, TKTD models have broader applicability for sub-lethal endpoints, assessment of multiple stressors and reverse dosing for high-throughput toxicity testing. Reviewing the state of the science of TKTD modelling will enable development of a roadmap towards wider use of TKTD models in environmental risk assessment of chemicals in general and highlight synergies and differences with human safety evaluation methods.

WE061
A mechanistic modelling approach to link mortality and feeding inhibition
A. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change; K. Ladermann, T. Knautz, gaiac - Research Institute for Ecosystem Analysis and Assessment; M. Hammers-Wirtz, Research Institute gaiac; S. Classen, gaiac Research Institute for Ecosystem Analysis and Assessment
Aquatic effect assessment is based on survival data obtained from acute laboratory experiments, and can be complemented by observations of sublethal effects at tier-1 or higher tiers. In particular, an individual organism's feeding rate is considered a sensitive endpoint with direct relevance for higher biological levels. Both mortality and feeding inhibition are measurable within relative short time spans, but experimental durations might differ among endpoints; e.g. a 48h acute toxicity test is compared with the results derived from 5 minutes feeding inhibition assay. Also, the long-term implications of these tests often remain unclear. Mechanistic effect models allow more informed effect predictions for the environmental risk assessment of chemicals through e.g. the extrapolation of untested situations or the integration of various study results. Toxicokinetic-toxicodynamic models take the process leading to an effect into account and, thus, are particularly suitable for the short-to-long term extrapolation of toxicity results. Here, we integrate the General Unified Threshold Model of Survival (GUTS) with a stress model for feeding inhibition to analyze toxicity data for various aquatic invertebrate species exposed to chlorpyrifos and to derive more general insights into the dynamics of mortality and feeding inhibition.

WE062
Temperature dependency of chemical effects predicted by physiological rate measures
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Aquatic effect assessment uses results from short-term laboratory experiments performed under constant environmental conditions. In contrast, natural populations regularly experience fluctuating environmental conditions, such as seasonal or diurnal variability in ambient temperature, which hampers a straight forward laboratory-to-field extrapolation. Moreover, comparing species sensitivity might be biased by experimental conditions as the different species are usually kept at different 'optimal' temperature regimes, which influences their apparent sensitivity. Here, we analyzed lethal effects on *Daphnia magna* caused by chlorpyrifos exposure at different ambient temperatures by means of the General Unified Threshold Model of Survival (GUTS). The model takes the process leading to an effect into account and describes mortality on the basis of internal concentrations or damage. Our results suggest that the temperature dependency of rate constants for damage accrual and effects can be predicted based on pyhsiologyical measures such as filtration rates or developmental rates. The suggested temperature extention to GUTS will allow the simulation of toxicity under more natural conditions and will facilitate the cross-species comparision of sensitivities.

WE063
Toxicokinetic-toxicodynamic model predictions of the survival of two aquatic macroinvertebrates for a mixture of an insecticide and a fungicide compound
A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; D. Belgers, M. Boerwinkel, L. Buijse, Wageningen Environmental Research / Environmental Risk Assessment Team; I. Roessink, Alterra / Environmental Risk Assessment Team; R. Osman, Wageningen Environmental Research / Environmental Risk Assessment Team; P. van den Brink, Alterra and Wageningen University
Toxicokinetic-toxicodynamic (TK/TD) models provide the means to mechanistically link time-variable exposure patterns to effects on the survival of a group of individuals. They are thought to be useful for the risk assessment of plant protection products, where predictions of mortalities can be calculated for quite large numbers of exposure scenarios. However, the TK/TD models need to be tested on performance before they can be applied for regulatory risk assessment. For a more realistic risk assessment for chemicals, it is also of basic interest to test mixtures of chemicals, because in the field organism are confronted not only to single chemicals, but to mixtures of chemicals of different source. In this study, data sets were generated that can be used for the calibration and the validation of TK/TD models for the insecticide cypermethrin and the fungicide fluazinam and two aquatic invertebrate species. Survival under 4 days constant exposure was monitored for the water louse *Asellus aquaticus* and the mayfly larvae *Cloeon dipterum* for the single compounds. Validation data sets were generated by testing two different time-variable exposure patterns on their effects on the survival of the two species, alone and in combination, in tests of 11 and 14 days duration for *C.dipterum* and *A.aquaticus*, respectively. The data showed that the timing of the exposure had only little influence on the final mortalities, and effects of the two compounds were adding up. These observations could be captured by the TK/TD model predictions, based on data from static tests with the single compounds. Hence, this exercise shows that TK/TD model predictions can be used to predict mortalities for more than one compound assuming simple mixture toxicity.

WE064
Modelling the toxicity of organic chemicals on aquatic invertebrates: maximizing applicability based on minimal data
A. Focks, Alterra Wageningen University and Research Centre / Environmental Risk Assessment Team; P. van den Brink, Alterra and Wageningen University
Recent advantages in the field of ecological and ecotoxicological modelling enable to link effect modelling to results of exposure modelling or to monitoring data. Such developments will help to improve the realism of environmental risk assessment by accounting for spatio-temporal variation in exposure patterns of chemicals rather than using constant exposure proxies such as 90^{th} centile or average concentrations. In order to expand ecotoxicological modelling also across the dimensions of species and chemical diversity, toxicity needs to be estimated for combinations of species and compounds beyond the standard test species, and also beyond the data rich chemicals, e.g. belonging to pesticides. This contribution reports on the formulation and parameterisation of parsimonious toxicity modelling approaches. Starting from the concept of toxic units, most simple one parameter models are formulated based on EC50 values, approximating lethal and sub-lethal effects when only one toxicity measurement is available for a species-compound combination. In case additional measured values are available per endpoint (e.g. EC10 or EC90 values), two-parameter log-logistic models can be used. One of the most comprehensive ecotoxicological databases (E-Tox database of RIVM/ NL) is exploited to provide toxicity model parameters for aquatic invertebrate species. The results are analysed in different ways: 1) an overview about total numbers of available toxicity parameters for different endpoints is given; 2) available toxicity model parameters are analysed more specifically for a choice of focal species for the Netherlands; 3) the available toxicity parameters are analysed for their correlations to chemical

properties and species traits. Based on the correlations, regression models for the prediction of toxicity parameters are tested. The parameter extraction and analyses are in progress. Currently, it appears that from the total number of >30.000 entries for aquatic invertebrates the major part uses mortality as an endpoint, and that for most combinations of species and compounds only one-parameter models can be parameterised. For the focal species, the information is thinning out when assessing non-standard test species, e.g. while for *Daphnia magna* EC50 values for mortality are available for >1.000 substances, for *Gammarus pulex* this data is available only for 48 and for *Asellus aquaticus* only for 39 compounds.

WE065
EasyGUTS - Running R GUTS scripts in a window based software
D. Nickisch, T. Lutz, Rifcon GmbH
We developed a Windows® software to manipulate and run R GUTS scripts for the evaluation of surface water exposure profiles calculated with the environmental fate tool FOCUS TOXSWA. Such software tools are very helpful and established for modelling environmental exposure of plant protection products in a regulatory context and finally for the decision making. For instance the common groundwater exposure model Pearl v 1.1.1 published in 2000 is nowadays accepted in all European Member states. However, within ecotox modelling approaches – despite the fact that they are mentioned as potential useful options in several guidelines - are still not commonly used or well accepted if developed within the European registration framework for plant protection products. Main point needs to be that model results have to be understandable and reproducable for all involved parties to increase acceptance of such models to be commonly used as a higher tier option in ecotoxicological risk assessement, and as actually intended also by EFSA. It is obvious that one person cannot be familiar with all available development environments like known Netlogo, Matlab or R, which are often used by ecotoxicologists. Since we are living in a ‘window dominated world’ the smallest common denominator is a window-based program running under Windows® operating systems. The other side of the coin is that a Windows® programmer, beside the computing skills, must have a deep knowledge in natural science and should best case be familiar with the regulatory framework. A modelling tool in higher tier aquatic risk assessments can be the toxicokinetic and toxicodynamic **G**eneral **U**nified **T**hreshold model of **S**urvival (GUTS) – similar to body burden modelling approaches in higher tier wildlife risk assessments. However, the first published GUTS version was written in R, a command-line program using an own script programming language, which is quite difficult to be used by persons which are not familiar with R. We developed a user-friendly Windows® program in the modern programming language VB.Net, which can run GUTS R scripts for parameterisation and validation of aquatic laboratory tests. Moreoever, we implemented an additional functionality which link the outcome of GUTS with exposure files from the environmental fate tool TOXSWA. The user can easily extract the exposure profiles from TOXSWA text outputfiles and perform a moving time window analysis to evaluate potential worst case exposure profiles.

WE066
Evaluation of effect models in the environmental risk assessment of plant protection products
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Environmental risk assessment (ERA) of plant protection products is carried out in a tiered process. Beside experimental refinement options to address the risk for non-target organisms identified at lower tiers (e.g. tests with modified exposure, more complex community studies such as micro-/mesocosm studies or field trials), mechanistic effect models have been increasingly submitted to authorities during the last years. Compared to experimental field studies, models are relatively inexpensive and can depict effects or scenarios that would be difficult to investigate in the field. Modeling has been identified as an appropriate methodology for "higher tier" refined risk assessment in the EFSA Opinion on Protection Goals (EFSA, 2010) as well as in various SETAC workshops. Despite the uncontested potential of this approach to address important issues in the risk assessment of pesticides, and despite the use of these models in research science, their implementation in the environmental risk assessment of PPP implies an evaluation of their quality and uncertainty in the identification and prediction of risks. For this, the relevant mechanisms that affect the sensitivity of different biological organization levels need to be known and then considered in the model. To generate confidence in the model predictions, the models (and each model application) must be evaluated using a set of quality criteria, prior to application in ERA. This allows risk assessors to weight the results of a model analysis in comparison with other lines of evidence. In a project funded by the German Ministry of Environment, Nature conservation, Building and Nuclear safety (BMUB), a critical evaluation of the suitability of effect models for their use into ERA is performed. Preliminary results indicate that most models lack validation through testing of predictions with independent data. In order to quantify the level of confidence that can be placed in the outcome of current risk assessment models, we will evaluate selected models spanning the range from lower to higher tier, as well as from individual to community/ecosystem level, according to the EFSA guidelines on good modelling practice.

WE067
How to address the complexity of environmental risk assessment - calling for a modular approach instead of large black box models
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Natural ecosystems are characterised by a high spatial and temporal variability and in recent years environmental risk assessment has begun to address this variability more and more realistically. However addressing this temporal and spatial explicit dynamics realistically is a very complex task, which needs expert knowledge from totally different disciplines, namely agricultural practice, chemistry, hydrology, geography, geology, ecotoxicology and ecology. Current model developments in this area are often very detailed in the aspect of primary interest of the developer whereas other processes are simplified, lumbed or even ignored. On the other hand complex single application models get intransparent by historic growth and increasing size of source code and come with an overwhelming complexity which cannot be judged by single experts. This fact automatically increases the mistrust and uncertainty in using these complex models. However models do not have to be developed from scratch. All models needed for environmental risk assessment could be built from a set of standardized more or less generic building blocks which would lead to a modular model development. Than model development and model validation can be conducted first on specific modules by experts in their field. This would allow that experts agree on a module in their field of expertise and later on it has only to be assessed if this module were built together in a meaningful way. This modules can be either linked hardcoded or in a much more flexible interface environment which might link components that may come from different suppliers, represent data and processes from different domains, be based on different concepts, have different spatial and temporal resolutions and representations. We will present our experience with this kind of modular approach.

WE068
Modelling laboratory standard biotests with Daphnia magna
T. Strauss, Research Institute gaiac / gaiac - Research Institute for Ecosystem Analysis and Assessment; T. Preuss, Bayer Ag / Environmental Safety
Acute and chronic tests with the water flea *Daphnia magna* (*D. magna* reproduction test, OECD 211; acute immobilization test, OECD 202) are standard tools in the ecotoxicological risk assessment for the aquatic environment. However, even standardized studies have often been carried out under different conditions (e.g. static, semi-static, flow-through or changes in guidance documents) and thus are difficult to compare in detail, because of different feeding regimes and exposure conditions. We present a modular modelling approach to analyse and compare results of these laboratory tests. Ecophysiological processes such as growth and reproduction were simulated with the individual-based population model IDamP dependent on experimental conditions, whereas mortality was described by the TK-TD modelling framework GUTS. With these models, reproduction per surviving individual can also be simulated as well as the reproduction per tested animals as used in the reproduction test (OECD 211), if the mortality over time is integrated. This approach allows the re-evaluation of all available laboratory tests, which were conducted under different feeding and/or exposure conditions. Multiple data sets for the same test compound can be used for model validation as well as for data analysis with regard to data consistency and consequently reduce uncertainty of risk assessment. Based on a broader data basis for validation by combining all available test data, this model approach allows to justify the time-weighted average (TWA) approach or to extrapolate effects to new exposure scenarios (peak or chronic exposure) if TWA cannot be applied.

WE069
Harpacticoid copepods in risk assessment - Combining life cycle experiments with population modelling
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While acute and chronic toxicity tests on the individual level are usually performed as a first step to assess chemical toxicity in environmental risk assessment (ERA), the protection goal in ERA is the population and community level. Combining life cycle experiments with population modeling can be a cost effective and powerful approach to extrapolate from individuals to populations. Population models based on dynamic energy budget (DEB) theory have been successfully applied to analyse stressor effects in terms of their energetic modes of action (MoA) on various invertebrate species in the past. These models can also be used to make predictions on population dynamics at scenarios of varying environmental conditions and toxic stress. Over the past few decades, copepods have gained increasing popularity as

invertebrate model organisms in ecotoxicology. As most crustaceans, they undergo sexual reproduction and thus form a welcome alternative to the still more commonly used parthenogenic *Daphnia*. Harpacticoid copepods form an important trophic link between the microphytobenthos in the sediments and higher trophic levels and thus are of high relevance for aquatic ecosystems. Unfortunately, only few attempts have been made to parameterise a DEB model for (harpacticoid) copepods due to some characteristic life cycle peculiarities that distinguish copepods from most other animals. In this study, the harpacticoid copepod *Nitocra spinipes* was chosen as a model species. In an individual based modelling approach (IBM) the DEBkiss model was used to build a mechanistic population model for this species. Slight modifications to the generic model structure were made to account for specific assumptions that were made for the copepod life cycle. These include a change in shape during the moult between the sixth naupliar and the first copepodite stage as well as an abrupt stop in growth after reaching the adult stage. The model was parameterised on life cycle data from literature and additional experiments. Using data on growth and reproduction over time at different temperatures and food concentrations we were able to predict ingestion and respiration rates. Furthermore, full life cycle experiments were performed with the antidepressant citalopram. The DEBkiss IBM was used to analyse this drug's toxic MoA on *N. spinipes*.

WE070

DEB(tox) models - Can we predict under different environmental conditions?

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The lack of ecological realism in current prospective environmental risk assessment is widely recognised as a limitation in this field. As organisms are living in a multistressed environment, involving both chemical and environmental stressors, it is worth understanding how these combined stressors will affect the organisms and subsequently the populations. A way forward to include more ecological relevance in ERAs is the use of environmental scenarios that will represent key differences in environmental factors such as the food availability, the temperature variability, the predation, etc. and in exposure factors. All these factors will influence the capability of an organism to grow and reproduce as well as its resilience to additional stressors. As growth and reproduction are driven by an organisms' energy balance, Dynamic Energy Budget models are particularly well suited to integrate toxicant and environmental stressors. Indeed, the DEB theory analyses the fluxes of energy within an organism, how stressors can impact these fluxes, and how this will affect the organism's life history traits. In this project, we assessed how DEB modelling can predict effects of various chemicals in variable environmental conditions. To do so, we produced two set of data for each chemical. In the first one, *Ceriodaphnia dubia* individuals fed ad-libitum and were maintained at reference temperature (25°C). In the second set of data, *C. dubia* individuals were maintained at a different temperature and feeding regime. For the purpose of this exercise, this second set of data was not disclosed to the team in charge of the DEB modelling. We used the first set of data only to calibrate the DEB model for *C. dubia*. Then knowing only the experimental conditions of the second set, we performed a blinded prediction of the growth, reproduction, and survival of *C. dubia* under environmental conditions that have not been used for the calibration. Blinded predictions and datasets were then compared and the ability of the DEB model to handle and accurately predict non-tested environmental conditions (temperature and feeding level) was analysed and discussed.

WE071

Population Models for Use in Baynesian Networks and Adverse Outcome Pathways

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We are developing holistic risk assessment models for the impacts of pollutants on aquatic species in four Puget Sound Basin watersheds: The Lower Skagit River, Lower Yakima River, Cedar River, and Nooksack River. These models are framed as Bayesian Networks (BN), probabilistic graphical models which allow random variables (represented by nodes) to be connected based on their conditional dependencies. Recently, BNs have been implemented in regional scale risk assessments because of their ability to incorporate multiple stressors to evaluate risk to desired endpoints, incorporating environmental variables that often confound traditional risk assessments. The BNs in this study will be based around established Adverse Outcome Pathways (AOPs), which function to link molecular initiating events (the direct physiological impact of a given pollutant on an organism) to population-level impacts by linking them through scales of increasing biological complexity. In this talk, I will present demographically-based, spatially explicit, matrix population models developed for Pacific salmon and show how data from these models are incorporated into BNs and AOP's to develop ecological risk assessments for organophosphate and carbamate pesticides, singly and as mixtures. We anticipate that the results of this study will be adaptable management tools that can be applied to organisms within any environment that experiences toxic impacts from chemicals that induce a predictable molecular response.

WE072

Conceptual model for implementing community model output in Ecological Risk Assessments of chemicals

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In recent years an increasing number of mechanistic effect models addressing higher levels of biological complexity (i.e., population and community) have been developed and documented in the scientific literature. In many cases the contemplated aim of these models is to use their output for higher tier Ecological Risk Assessment (ERA). Such models may be used as virtual laboratories where conditions may be changed and simulations re-run repeatedly at relatively low cost once the models are developed and thoroughly tested. Based on effects measured at the individual level and combined with realistic exposure scenarios, population and community models may thus provide a flexible tool for predicting risk for ultimate protection goals. Despite these prospects and anticipated benefits, the practical use of such models in ERA is still only in its infancy, and the majority of examples of actual and suggested use are specifically related to ERA of plant protection products (PPPs). In this presentation we present a community model under development as a visual representation of major components and processes, as well as the first draft of a conceptual model for how output from the community model may be integrated into a REACH type legislative framework. The aim is to use this conceptual model and the presentation to initiate a stakeholder consultation process. In this consultation process, we wish to discuss the envisioned use of community model output for ERA of chemicals other than PPPs with relevant stakeholders and the feedback received will be considered and possibly implemented in the final version of the framework.

WE073

The use of population models in copper risk assessment: a case study with Lymnaea stagnalis

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Conventional metal risk assessment is based on metal bioavailability and species sensitivity distributions (SSD). An ever increasing number of studies on chronic metal toxicity are being published. Furthermore, the SSD is based on toxic effects that occur at the individual level. In the real environment, however, we want to protect populations and communities within the ecosystem. Modelling techniques in ecological risk assessment are becoming increasingly more prominent. They can be used to predict long-term effects of chemicals and other stressors on population dynamics. In this study a population model was developed, integrating available data on various endpoints of copper toxicity on the pond snail, *Lymnaea stagnalis*. Effects of copper on freshwater snail survival and growth rate (*Lymnaea stagnalis*) have been demonstrated, as well as effects on reproduction and embryo development (*Lymnaea luteola*). As a result of these laboratory experiments the adverse effects of copper have been quantified. Data on copper dose-response relationships is available, and can thus be incorporated in mechanistic models to extrapolate copper effects from the individual to the population level. The proposed model is an individual based model (IBM) of the dynamic energy budget theory (DEB), adapted for *Lymnaea stagnalis*. IBM describes population dynamics through individual behavior, while DEB describes physiological processes on an individual level. DEB-IBM can therefore simulate the impact of stressors on individual animal processes (assimilation, maintenance, reproduction, growth, survival) which can then be projected to the population level. Simulations with the Lymnaea DEB-IBM indicate a decrease in population growth rate and carrying capacity with increasing copper concentrations. Furthermore, there is a shift in age structure between snail embryos, juveniles and adults. Effects on population reproduction and mean adult snail size are predicted as well. Based on simulations with a range of copper concentrations, population level NOECs and EC10s can be derived, and this for varying environmental conditions (e.g. food availability) and different population endpoints. Comparing these values to individual level NOECs and EC10s, can help steer legislation towards a population-level protection of the environment regarding copper pollution. This study addresses the importance of mechanistic population models as tools to improve ecological relevance in (copper) risk assessment.

WE074

Modeling the interactions between Daphnia growth and algal density under different environmental conditions using Dynamic Energy Budget theory

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Understanding the uptake dynamics of *Daphnia* is important to expect the dynamics of phytoplankton populations and food-web structures in the aquatic systems. In this study, laboratory experiments and modeling study was conducted to explore the density dynamics of freshwater algae species, *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*, and *Daphnia magna* growth under various temperature (15, 20, 25, and 30°C) and copper exposure (5 and 10 μg/l) in mono- and mixed algal cultures. A Dynamic Energy Budget (DEB) model was used to simulate the *D. magna* growth by algae uptake, and algal density model was combined into DEB in System Dynamics Model (SDM) interface to describe the algal density dynamics over time. Our results showed that *D. magna* growth strongly depends on algae density which affected by tested conditions, and is less affected in mixed algal cultures than in monocultures. Modified DEB model by observed data well describe the interactions between algal density and *D. magna* length under various environmental conditions. The present approach can be utilized to combine the DEB and the other developed model for exploring the complex ecological interactions in ecosystems.

WE075
SETAC Interest Group on Mechanistic Effect Models for Ecological Risk Assessment
U. Hommen, Fraunhofer IME

Highly Hydrophobic Chemicals: Reliable Investigations on Environmental Fate and Effects (P)

WE076
Application of passive dosing in the standardized aquatic toxicity test of hydrophobic organic chemicals for regulatory chemical assessment
H. Watanabe, M. Noguchi, N. Tatarazako, H. Yamamoto, National Institute for Environmental Studies / Center for Health and Environmental Risk Research
Maintaining the saturated concentration of poorly water-soluble chemicals (i.e. hydrophobic organic chemicals) during aquatic toxicity tests remains a technical challenge in the testing laboratories. OECD Guidance Document No.23, which suggests the recommended methods for testing difficult substances and mixtures, allows to use carrier solvent (e.g. dimethylformamide, dimethyl sulfoxide) at 0.1mL/L (100 mg/L) or less. Even though 0.1 mL/L solvent is not toxic to test organism, it may alter physiological status of test organisms and cause enhanced or offset toxicity of test chemical. To address these problems, passive dosing has been recently under reviewed as a new method in the draft updated OECD Guidance Document No.23. Passive dosing uses the polymer, which is first loaded with the test chemical and then applied as a partitioning donor that controls exposure concentrations throughout the test. Several previous experiments demonstrated the polymer such as C18 and polydimethylsiloxane (PDMS) successfully provides the exposure of hydrophobic organic chemicals such as polychlorinated biphenyl (PCB) and polycyclic aromatic hydrocarbons (PAHs) at the solubility limit (Mayer et al., 1999, Smith et al., 2010; Adolfsson-Erici et al., 2012; Seiler et al., 2014;). However, in the view point of regulatory framework of chemical risk assessment, there is limited validation study applying passive dosing on traditional standardized test methods such as OECD test guidelines (e.g. TG201, 202, 203, 210, 211). Especially, practical problems of passive dosing during long-term test using flow-through exposure system remains unsolved. In this study, first, we tested PDMS polymers in the OECD acute toxicity tests of hydrophobic organic chemicals (e.g. PAHs) and compare the results with traditional methods (carrier solvent and “water associated fraction”). Second, we used PDMS column in the flow-through exposure system to confirm the capacity of the column to maintain test chemical at saturated level during fish long term toxicity test (2 weeks, at least).

WE077
Passive dosing for the study of hydrophobic environmental contaminants CPs in ecotoxicological studies
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Chlorinated paraffins (CPs) are a group of industrial chemicals consisting of n-alkanes (10 to 30 carbon chain atoms) with varying chlorine content.These compounds are ubiquitous environmental contaminantsbecause their global production increased greatly in the last decades due to the high usage as high-temperature lubricants, flame retardants, and additives in adhesives, paints, rubber, and sealants.In Europe, short chain chlorinated paraffins’ (SCCPs) use has been restricted: these compounds are considered both PBT- (i.e., persistent, bioaccumulative and toxic) and vPvB classed (i.e., very persistent, very bioaccumulative) substances. However, medium (MCCPs) and long chain (LCCPs) chlorinated paraffins are still used as substitutes for the SCCPs in Europe and outside. Moreover, in some countries (e.g., China), all CP classes are still in use, leading to high production, usage and release to the environment (up to a million tons per year). Analysis of CP-52 (labelled as a MCCP) produced by Chinese manufacturers, has identified that this is a mixture of SC and LCCPs congeners, raising concerns as this is the most commonly found CP in China. The lack of data concerning this group of contaminants is due to the methodological difficulties in analytical identification as well as ecotoxicological testing due to their high hydrophobicity. Passive dosing offers a method to overcome problems related to losses of the test substance due to sorption, volatilization and degradation. To create a controlled and stable exposure system for studying effects of exposure to CPs, we apply a passive dosing system consists of silicone (PDMS) loaded with CPs. When brought in contact with the exposure medium, an equilibrium will be established. Using analytical quantification methods (APC-QTOF-MS and GC-MS), we quantified different mixtures of CPs (2 SCCPs, 1 MCCP, 1 LCCP and CP-52) in both water and silicone phase. This work helps to understand the differences in behavior and partition between different groups and congeners of CPs. Since chlorinated paraffins have the potential to severely harm exposed biota (both aquatic and terrestrial), this system can be used to fill knowledge gaps, helpful not only for the scientific community, but also current gaps in the regulatory field. Future applications/studies are generating ecotoxicological data on growth and survival in *Daphnia magna* exposed to different CPs and comparison of the risks and hazards between SC and MCCPS.

WE078
Passive dosing via the headspace: A simple method for controlling exposure of volatile hydrophobic organic compounds in aquatic toxicity tests
L.N. Trac, Technical University of Denmark / Environmental Engineering; S.N. Schmidt, P. Mayer, Technical University of Denmark / Department of Environmental Engineering
Several passive dosing formats have been developed to control the exposure of hydrophobic organic compounds (HOCs) in experiments investigating their toxicity and speciation. The first step is generally the thorough cleaning of a silicone elastomer, which is then loaded with the test substance before usage as partitioning donor for (1) establishing test substance concentrations and (2) maintaining the exposure by replenishing losses due to sorption, (bio)degradation and volatilisation throughout the test. In the present study, a simpler passive dosing method was developed for volatile hydrophobic organic compounds, and subsequently applied in limit tests that were operated exactly at the saturation level. In this new passive dosing system, the test compounds were dosed from their pure phase contained in a glass insert via the headspace to the aqueous test medium. The test model compounds were n-alkanes with the number of carbons ranging from 9 to 14, i.e. n-nonane, n-decane, n-undecane, n-dodecane, n-tridecane and n-tetradecane. The new method was used to control exposure exactly at the saturation level, which was then confirmed by headspace gas chromatography and cross validated by passive dosing using saturated silicone rods. The results confirmed a high dosing efficiency of this new and simpler technique as well as its applicability to aquatic toxicity testing. Finally, some initial toxicity limit tests were performed with the green algae *Pseudokirchneriella subcapitata* using the new dosing approach to assess the toxic effect of the test model compounds at the saturation level.

WE079
Challenges of the Aquatic Risk Assessment of Pyrethroids: A Case Study for the Insecticide Deltamethrin
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Pyrethroids are a group of plant protection products with excellent efficacy against insect pests. Due to their high activity there is a risk of unintended ecological side-effects, in particular on aquatic organisms, which requires a detailed aquatic risk assessment. However, the highly hydrophobic nature of pyrethroids strongly influences their behaviour in the environment, poses significant challenges for the planning, conduct and interpretation of experimental studies, and introduces uncertainties in the aquatic risk assessment. We will highlight the most important of these challenges and will propose solutions for the example of the pyrethroid insecticide deltamethrin. Due the strong sorption of deltamethrin to organic material and surfaces in general, suspended solids strongly affect dissolved concentrations and bioavailability of the compound. This has direct consequences for the interpretation of observed ecotoxicological effects, which must always consider the actual exposure situation in the experimental study. On the other hand, model calculations of deltamethrin exposure concentrations in water and sediment are also challenging because many models have upper limits for the input of sorption parameters. For a reliable, robust aquatic risk assessment of deltamethrin it was necessary to review and partly adapt standard assumptions and model settings. The conceptual work and wealth of data collected by the Pyrethroid Working Group (PWG) in the U.S. was highly useful in this context.

WE080
Understanding Bioavailability of Pyrethroid Insecticides in Aquatic Environments
J. Gan, University of California, Riverside / Department of Environmental Science
Synthetic pyrethroids are an important class of contemporary insecticides used in

both agricultural pest management and public health protection. Pyrethroids are extremely hydrophobic with log K_{ow} in the 5-7 range and most are sparingly soluble in water. Pyrethroid compounds are potent neurotoxins and incite acute toxicity to aquatic invertebrates at the sub-ppb level. Because of their strong hydrophobicity, the fate and aquatic toxicity are driven closely by bioavailability. Our group has carried out comprehensive investigation on the bioavailability of pyrethroids in aquatic environments, including understanding their mode of transport from terrestrial to surface aquatic systems, development of solid phase microextraction (SPME) and polyethylene film-based passive samplers for detecting the freely dissolved concentration (C_{free}) in surface water and sediment porewater, and correlation of C_{free} with bioaccumulation and toxicity endpoints for aquatic invertebrates. Pyrethroids are transported off-site as attachment to fine particles in surface runoff. Sorption to dissolved organic matter or solids consistently inhibited the bioavailability of pyrethroids, leading to reduced uptake or acute toxicity. Organic matter characteristics and aging further influenced this process. This presentation will provide an overview of our current knowledge on the bioavailability of pyrethroids and highlight information gaps warranting future research.

WE081

Spatial distribution of POPs in fish from Argentina: relationship with the surrounding land uses

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Persistent Organic Pollutants (POPs) are lipophilic, persistent and globally distributed compounds that bioaccumulate in fish. Aquatic environments are subject to poisoning risks due to the inputs of these contaminants from agricultural, urban or industrial activities. The aim of this study was to investigate the POPs distribution in teleost and chondrichthyan tissues and its relationship with the surrounding land uses. Fish were collected in three different areas of Argentina: I. the Negro River basin (41º56'S-72º29'W). *Cyprinus carpio*, *Odontesthes hatcheri*, *Oncorhynchus mykiss*, and *Percichthys colhuapiensis*) were sampled. II. Dulce creek basin (37º6'S-57º55'W) *Rhamdia quelen*), and III. Coastal waters of Southwest Atlantic (34º-41ºS) *Callorhinchus callorynchus*, *Sympterygia bonapartii*, and *Rioraja agassizii* were collected. Muscle, liver, gonads tissues were sampled in males and females.. Endosulfans (a-, b- and sulfate), DDTs (DDE, DDD, DDT), PCBs and PBDEs were determined by GC-ECD. Fish tissues from the Negro river showed the highest POPs levels (15-1200 ng/g ww) with enrichment in DDTs (45-81%, mainly DDE) while PCBs were found at lower levels. The distribution pattern of pesticide in those fishes could be mainly related to the huge fruit production area where DDT was applied during past decades, in addition to the presence of industrial centers and hydroelectric power plants, located around the Negro River basin. A similar pattern was found in other matrixes from the basin, such as soils, macrophytes and sediments. Catfish from Central-East region (5.5-17.2 ng/g ww) and chondrichthyans from Coastal waters presented a clear Endosulfans predominance (37-45% and 50-100%, respectively). These patterns observed in both regions are consistent with the widely soybean production area where this insecticide together with chlorpyriphos and cypermethrin are used. Despite the similar pattern, marine chondrichthyans showed lower POPs levels than catfish. It is clear that the impact of land use could be reflected in different distribution patterns of POPs in fish. This finding is more consistent when fish from freshwaters are analyzed where a direct impact is observed. Coastal marine fish receive a mixture of many contaminants and those persistent are found in different tissues. These results revealed that surrounding land uses (agriculture and urban-industrial settlements) to aquatic environments represent past and current sources of POPs to fish.

WE082

EPA's Expanded-List PAHs in Environmental Media

B.H. Magee, ARCADIS; N.D. Forsberg, ARCADIS / Environmental and Molecular Toxicology

In 2010, US EPA proposed Relative Potency Factors (RPFs) for 24 PAHs, thus increasing the list of potentially carcinogenic PAHs likely to be included in human health risk assessments from 7 to 24. This proposal has not yet been implemented by the US EPA and is still under internal review. The 17 additional PAHs are not routinely included in analyte lists for environmental sampling, so at the time the new RPFs were proposed little was known about their presence in the environment. In the intervening years, many have included some of the additional 17 PAHs, such as benzo(c)fluorene, benzo(j)fluorene, and cyclopenta(cd)pyrene, in their analytical programs. Some of these PAHs are present in a variety of environmentally relevant matrices, including atmospheric particulate matter, marine sediment, diesel exhaust, automobile tires, coal tar, urban dust, urban soil, various foods, and cooked meats. Data from the authors and from published literature will be presented, and the increase in total PAH concentrations as well as total benzo(a)pyrene toxic equivalent concentrations will be calculated and presented relative to current PAH risk assessment practices to show the impact of this proposed new list of PAHs on environmental decision making. In addition, a status report will be presented on EPA's actions on PAHs.

WE083

Passive sampling in kinetic mode: Determining dissolved concentrations by using the concentration ratio of passive samplers with different thicknesses.

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The use of nonpolar passive sampling devices is a common method to measure bioavailable concentrations of waterborne hydrophobic compounds in the environment. The rate at which an equilibrium between the receiving phase and water is attained differs depending on the hydrophobicity. For highly hydrophobic compounds, equilibrium attainment can take months. In those cases, the dissolved concentration in water has to be estimated by applying passive sampling devices in kinetic mode. When kinetic sampling is used, various influencing factors (e.g. water turbulence and varying temperature) need to be considered. This requires the use of performance reference compounds whose release rate is a measure of the sampler exchange kinetics. As PRCs are often expensive and not always available, other approaches would be helpful. To achieve this an experiment was conducted based on the hypothesis that when two samplers made of the same material and with the same surface area but different volumes are deployed in parallel in the same hydrodynamic regime, measuring the ratio of their concentrations at a single point during the kinetic uptake phase permits the dissolved concentration to be determined. To investigate this, silicone passive samplers with two different thicknesses and PAHs with a wide range of physico-chemical properties were chosen. At different points in time, thick and thin samplers were taken out, extracted and analysed. The dissolved concentrations were calculated for different points in time using the measured concentration ratios in the thick and thin samplers. These calculated concentrations were comparable to the measured dissolved concentration. This approach opens up an efficient possibility of determining environmental dissolved concentrations during kinetic sampling.

WE084

Measuring dissolved-phase hydrophobic halogenated flame retardants in the water of the US Great Lakes using polyethylene passive samplers

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Measuring dissolved-phase concentrations of hydrophobic organic contaminants (HOCs) is essential to understand the transport and fate of HOCs in aquatic environments, as well as their impacts on aquatic ecosystems. However, the dissolved phase is difficult to measure via traditional active sampling methods. In this study, polyethylene passive samplers (PEs) were deployed in surface waters throughout the lower Great Lakes in the United States from spring to fall of 2013 to measure time-weighted average concentrations of dissolved flame retardants and polycyclic aromatic hydrocarbons (PAHs). PE extracts were analyzed by GC/MS in negative chemical ionization (NCI) mode to measure mono- to octa-brominated diphenyl ethers (BDEs) and nine novel halogenated flame retardants (NHFRs). Extracts were also analyzed by GC/MS in electron impact (EI) mode to measure a suite of 2- to 5-ring PAHs and alkylPAHs. BDEs 15, 28, 49, 47, 100, 99, 154, 153, and 183 were found at >50% of locations. Σ_9BDE ranged from below detection limits at an offshore buoy in eastern Lake Erie to 41 pg/L near the shoreline of western Toronto in Ontario, Canada. Among the NHFRs, pentabromobenzene (PBBz), pentabromoethylbenzene (PBEB), hexabromobenzene (HBBz), and syn- and anti-Dechlorane Plus (ADP and SDP) were detected most often, with maximum dissolved Σ_5NHFR of 2.8 pg/L near the shoreline of Toronto. Σ_{27}PAH ranged from 0.3 pg/L near the rural shoreline of northern Lake Ontario to 52 pg/L on the shoreline near an urban airport. Here, spatial distributions of dissolved HOCs in the lakes and their relation to nearby point sources and urban centers will be investigated and Bayesian kriging will be used as a geostatistical interpolation technique to predict lakewide concentrations of dissolved species. Uptake of dissolved HOCs from water into polyethylene over time, and comparison to active sampling results, will also be discussed.

WE085

Sorption studies of polychlorinated biphenyls (PCBs) between aqueous solution and soil particle grain sizes: Effects of temperature and ionic strength.

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Introduction: The PCBs belong to the most persistent, bioaccumulative, and toxic organic pollutants that poses a threat to both human and aquatic environments (Gdaniec-Pietryka, *et al*. 2009). Due to the hydrophobic character of PCBs, they tend to partition onto suspended particulate matter (SPM) in water, soil organic matter (SOM) as well as fatty tissues of animals (Ericson, 2011). The distribution of organic pollutants, such as PCBs, in environmental media is mainly controlled by sorption-desorption process with various parameters affecting its partitioning between the water and sediment. The aim of this study was to investigate the effect of temperature and ionic strength in the partitioning of selected indicator PCBs between aqueous solution and various soil particle grain sizes. **<strong>Method**: The surface areas, pore volume and pore size distributions of the different soil particle grain sizes were determined using BET adsorption-desorption isotherms. The effect of temperature (283, 393, 398, 303 and 313 K) on the sorption experiments was carried out using the batch adsorption method with an equilibration period of 24 hours. The effect of ionic strength on the partitioning of PCBs between the aqueous solution and soil particle grain size was investigated using KCl as the background electrolyte. **<strong>Results**: The sorption capacity of PCBs positively correlated with soil organic matter (SOM) content, surface area as well as pore size distribution among the soil particle grain sizes.The result of batch experiments showed that the sorption capacities for PCB congeners varied with different soil particle grain sizes. The smaller soil particle grain size had the highest sorption capacity of PCB congeners compared to the larger soil particle grain sizes (75 μm > 100 μm > 200 μm > 425 μm > 300 μm). Low temperature had higher partitioning of PCBs onto the soil compared to high temperatures. An increase in the solution temperature probably caused an increase in the aqueous solubility of the PCB congeners thus leading to lower partitioning to soil at high temperatures. Thermodynamic studies showed that the partition coefficient (Kd) decreases with an increase in temperature. Concentration of the ionic strength was found to be less significant in the sorption of PCBs.

WE086

Quantification of PCB and PCDD partitioning constants between water and dissolved phases by polymer depletion techniques

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Determination of the partitioning behaviour of highly hydrophobic contaminants (hHOCs, log K_{OW} > 5.5), many of which can be highly toxic e.g. dibenzo-*p*-dioxins (PCDDs), in aqueous environments to dissolved phases is not straight forward. However, partitioning of hHOCs to dissolved materials such as surfactants can alter their behaviour by enhancing apparent aqueous solubility. Relevant partition constants are challenging to quantify with reasonable accuracy. Potential reasons may be the physico-chemical properties of hHOCs which result in slow kinetics and potentially relatively high losses to experimental surfaces. Furthermore, a phase separation of the dissolved phases and water to measure both phases individually without disturbing the equilibrium may not be feasable. Therefore, only limited data is available in the literature. Existing data shows an overall high variability. A mass balance approach in combination with a passive dosing technique was adapted to quantify hHOC partition constants to dissolved phases. The passive dosing of contaminants via a third phase (PDMS) into the test solution however relies on the known PDMS-water partition constants as well as a known added mass to the depletion system. Therefore, the polymer loading was modified from traditional methanol-water technique to a hexane swelling loading. We quantified partition constants of PCBs and PCDDs (log K_{OW} 5.8-8.3) between water and sodium dodecyl sulfate monomers (K_{MO}) and micelles (K_{MI}). A refined, recently introduced swelling-based polymer loading technique allowed highly precise (4.5-10% RSD) and fast (K_{MI} and K_{MO}. hHOC losses to experimental surfaces were substantial (8-26%) in monomer solutions, but had a low impact on K_{MO} (0.10-0.16 log units). Log K_{MO} for PCDDs (4.0-5.2) were approximately 2.6 log units lower than respective log K_{MI}, which ranged from 5.2-7.0 for PCDDs and 6.6-7.5 for PCBs. The linear relationship between log K_{MI} and log K_{OW} was consistent with more polar and moderately hydrophobic compounds. Apparent solubility increased with increasing hydrophobicity and was highest in micelle solutions. However, this solubility enhancement was also considerable in monomer solutions which may present a long term transport pathway for hHOCs in the subsurface due to monomers pseudo-persistance.

WE087

Measuring and Modeling Membrane-Water Partitioning Coefficients for Cationic Surfactants

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Cationic surfactants are important ingredients in various household products, personal care products and industrial processes. Many cationic surfactants are technical mixtures of single chain amines with hydrophobic alkyl chain lengths ranging between C_{12}-C_{18}, while also C_{22} compounds (behentrimonium) and several dialkylamines with C_{10}-C_{18} chain lenghts are common. As for many ionogenic compounds, the environmental fate assessment of cationic surfactants is complicated because it is not clear how to parameterize critical partition coefficients such as Kow, Koc, or how to model uptake in organismal tissue. It is expected that the membrane-water partition coefficient is driving the overall tissue-water partition coefficient, because membranes allow for both ionic interactions at the phospholipid head groups and hydrophobic interactions at the membrane core [1]. We determined the distribution coefficient between phospholipid membranes and PBS medium ($D_{MW,PBS}$) for 19 cationic surfactant structures, using an optimized solid supported lipid membrane (SSLM) assay, a chromatographic (IAM-HPLC) tool and molecular calculations for ionic structures (COSMOmic). The SSLM data set was used to construct QSARs for $D_{MW,PBS}$ofcationic surfactants, and to validate chromatographic capacity factors and molecular simulations of $D_{MW,PBS}$.

WE088

Environmental risk assessment of poorly soluble substances: Improved tools for assessing biodegradation, (de)sorption, and modeling (project RABIT)

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All chemicals that are supposed to be imported into the EU or put on the EU-market have to be registered at the European Chemicals Agency (ECHA) in Helsinki. Currently there are more than 850 substances with a log Kow ≥ 5.5 registered. They are used in a broad range of applications, ranging from consumer products to industrial applications. High production volumes and consumer application (e.g., cosmetics) may promote the potential release of substantial amounts of these chemicals into the wastewater and the aquatic environment. Due to their intrinsic properties, they have to be considered as potential PBT candidates. Investigations on environmental fate and toxicity of highly hydrophobic chemicals with an extremely low aqueous solubility are crucial but not straightforward. They always carry various difficulties in their procedures due to the chemical properties at hand. Sorption to solids such as soil matrices and accumulation in different phases strongly influence their bioavailability. As biodegradation is strongly dependent on (fast and slow) desorption of the test substance into the water phase, this can easily lead to false negative results: when desorption is slow, the biodegradation rate can be artificially decreased due to limited bioavailability. Thus, standard tests are often not suitable for these chemicals. In order to provide a more reliable assessment of the biodegradation behavior of highly hydrophobic chemicals, the aim of this project is to develop new experimental setups that can adequately decouple desorption effects from the actual biodegradation process. Additionally, the measurements on aqueous phase biodegradation and abiotic desorption will be coupled into a unified model. The project is funded and supported by the European Chemical Industry Council (CEFIC) in frame of the Long-Range Research Initiative (CEFIC LRI-ECO32), in cooperation with the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) and the German Federal Environment Agency (Umweltbundesamt).

WE089

Prediction of the Formation of Biogenic Non-Extractable Residues

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The fate and turnover of pesticides and other compounds is assessed in degradation tests with labelled molecules. Usually, a significant fraction of label is present as non-extractable residues (NER) at the end of the tests. Due to the mostly unknown speciation of these NER they are generally considered as potentially toxic parent compounds or metabolites in risk assessment [1]. Recent findings strongly indicate that a significant fraction of NER is formed from bacterial decay products, and within the microbial food chain. We present a new method for the prediction of biogenic NER (bioNER) formation. A key parameter for this prediction is the microbial yield, which is defined as the ratio of biomass formation to substrate consumption. Common methods for the calculation of yield, derived in biotechnology, are based on balancing the Gibbs energy of formation of products and educts. We developed a new pathway-independent method dedicated for the estimation of yields from xenobiotic substrate that includes the Nernst equation and the availability of electrons to biological processes. Labelled substrate turns into metabolites, biomass, non-extractable residues (NER) and CO_2. We have set up a dynamic model that simulates biomass formation from xenobiotic substrate, biomass turnover, and the formation of soil organic matter. It uses the common equations for enzymatic kinetics and microbial growth (Michaelis-Menten and Monod kinetics) and is expanded for the terms biomass decay, new biomass formation and non-living soil organic matter formation. The yield is pre-estimated, and for the decay rate default values can be used. Thus, only few fit parameters remain. The simulation results are contrasted to experimental studies. In such degradation experiments with labeled substances, bioNER is either the label within microbial biomass, or within soil organic matter. The amount of both can be predicted from the yield of the xenobiotic substrate and the CO_2-formation during the test. This allows to estimate bioNER formation in degradation tests, and, by subtraction, the quantification of sequestrated or covalently bound parent compound. Brief, the new method allows to differentiate between problematic, risky NER and harmless biogenic residues. [1] Kästner M, Nowak KM, Miltner A, Trapp S, Schäffer A. 2014. Classification and modelling of non-extractable residue (NER) formation of xenobiotics in soil. Crit Rev Environ Sci Technol 44, 1-65

WE090
Testing of viscous liquid mixtures
D. Thomas, Smithers Viscient UK
High viscosity liquid mixtures present a number of challenges for testing and chemical analysis, particularly for water solubility Log Pow and vapour pressure testing. Adaptation of the slow stir method cited in OECD 123 is useful for determining water solubility for liquids less dense than water. Over an extended period, up to four weeks it yields good results, mitigating the requirement for filtration due to the slow stirring speeds. Taking aspects from OECD 105 and OECD 123 has addressed the problem of water solubility of liquids more dense than water. This utilises a fast stirring speed, and a period of time without stirring and using turbidity to check for particulates. Performing Log Pow using slow stir (OECD 123) produces very good results for a Log Pow in excess of 3. Assessing the Log Pow of surface active materials by this method however, is still problematic, which was visually illustrated when a highly coloured substance was analysed. Two two layers formed in the octanol phase with nothing detected in the aqueous, even though the water solubility was 100 μg/mL. This would suggest that slow stir cannot be reliably used for determination of the Log Pow of a surface active material. Contrary to what is suggested in OECD 104 measuring vapour pressure of a mixture by mass effusion can be performed for vapour pressures up to 10 Pa and as low as 1×10^{-5} Pa. In practice it is the vapour pressure of the most volatile component that is measured. The proportion of the most volatile component present in the sample will determine whether the optimum approach is to use the same sample at different temperatures or fresh samples.

Organic micropollutants in the environment: analytical challenges and engineering innovations (P1)

WE091
Low-cost pharmaceutical removal from water using activated carbon prepared from cherry/sweet cherry kernels
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In recent years, the problem of contaminants such as pharmaceuticals and personal care products (PPCPs) in wastewater has increasingly attracted public and scientific interest. When surface water becomes contaminated with these compounds, aquatic life can be harmed, even by trace amounts. As a consequence, a number of wastewater treatment plants have to upgrade their technologies by introducing an additional PPCPs removal process. In present study, removal of six pharmaceuticals from aqueous solutions was done using cost-effective engineered adsorbent. The precursors for activated carbon were lignocellulosic raw materials (sweet/sour cherry kernels), as industrial byproducts and components of organic solid waste. Activated carbon synthesis was carried out by thermochemical conversion (H_3PO_4, 500 °C) in the complete absence of inert atmosphere. Obtain eco-friendly activated carbon (CScPA) showed huge uptake of pharmaceuticals. Therefore, CScPA was studied in detail by characterizing the material and investigating the adsorption performances. The effects of pH, adsorbent dosage, contact time and initial concentration of sulfamethoxazole, carbamazepine, ketoprofen, naproxen, diclofenac and ibuprofen on adsorption were studied in a batch process mode. In order to assess the multicomponent effect on removal efficiency, adsorption of all six pharmaceuticals was conducted simultaneously. Langmuir isotherms give a better fit than the Freundlich isotherms, except for ibuprofen, revealing that the adsorption was mainly monolayer. The monolayer adsorption capacities, q_{max}, calculated from Langmuir model were 19.18, 21.90, 19.67, 20.55, 21.46 and 20.54 mg/g for sulfamethoxazole, carbamazepine, ketoprofen, naproxen, diclofenac and ibuprofen, respectively. Adsorption kinetics are followed by pseudo-second order model, which implies that adsorption for all pharmaceuticals was mainly chemisorption process. Performed study showed that activated carbon prepared from cherry/sweet cherry kernels is useful adsorbents because it is cheap, recyclable and highly efficient. According to obtained results, the CScPA was found to be a promising low-cost adsorbent for the removal of investigated pharmaceutical from contaminated water. **Acknowledgement:** Ministry of Education, Science and Technological Development, Republic of Serbia (III46009) has financially supported this research.

WE092
Applying high-resolution mass spectrometry (UHPLC-Orbitrap-MS/MS) to evaluate the efficiency of wastewater treatment
Z. Li, Stockholm University / ACES; M. McLachlan, Stockholm University / Environmental Science and Analytical Chemistry (ACES)
During the past decade we have witnessed an explosion in the number of micropollutants reported in surface water. Many of these micropollutants are reaching surface water due to their incomplete removal with conventional wastewater treatment technologies. Although the removal efficiency of micropollutants in wastewater treatment plants (WWTPs) is a major determinant of their environmental impact, measurements of removal efficiency are available for only a few pollutants. The implementation of liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) for screening of organic micropollutants in aquatic systems opens new possibilities to address this knowledge gap. Here we present a process-oriented non-target method to evaluate wastewater treatment efficiency. Flow-proportional influent and effluent samples (24 h) were collected from the Henriksdal municipal WWTP in Stockholm, Sweden in June 2016. The samples were spiked with a mixture of 45 isotopic-labelled internal standards of a wide group of polar pollutants (mainly for quality control purposes). After filtration, the samples were analyzed with UHPLC-Orbitrap-MS/MS using direct injection. The subsequent data processing workflow included peak picking, blank subtraction, monoisotopic/isotopic peak clustering and adduct peak assignment; all steps were carried out with open-source R packages ("enviPick", "enviMass", and "nontarget"). The workflow was applied simultaneously to WWTP influent and effluent samples to demonstrate the potential of the non-target analysis for process evaluation. Briefly, by drawing a Venn Diagram based on the components detected in the influent and effluent samples, three categories of pollutants are possible: i) components detected only in influent, which indicates efficient removal during the wastewater treatment; ii) components detected only in effluent, which indicates that they were generated during the wastewater treatment; and iii) components detected in both influent and effluent, which indicates incomplete removal during the wastewater treatment processes. For those components with high abundance from the last two categories, identification using chemical databases (e.g., PubChem and ChemSpider) will be performed to generate a priority list of pollutants.

WE093
Influence of Cambi Thermal Hydrolysis/Anaerobic Digestion Treatment on Concentrations of Plasticizers in Wastewater Sludge
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Plasticizers are organic compounds commonly used to improve the flexibility of plastics, such as polyvinylchloride (PVC). Use of these compounds in variety of products, including PVC piping, has led to their detection in the wastewater treatment process, where they largely associate with solids. As such, plasticizers can be introduced into the environment via the land application of biosolids, drawing concern due to their toxicological and bioaccumulatory properties. This study focuses on the fate of 5 plasticizers [bis(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), benzyl butyl phthalate (BBP), and bis(2-ethylhexyl) adipate (DEHA)] during wastewater solids treatment via the Cambi Thermal Hydrolysis Process™ in conjunction with anaerobic digestion (TH-AD). Concentrations of the target analytes in biosolids produced by the TH-AD process, sludges from the individual TH-AD treatment steps, and limed biosolids not treated by TH-AD from the same wastewater treatment plant were compared. Concentrations of DEHP, DiNP, and DiDP significantly increased during the anaerobic digestion stage of the TH-AD process while BBP and DEHA both increased due to thermal hydrolysis treatment but decreased during the anaerobic digestion stage of treatment. DEHP, DiNP, and DiDP levels in limed biosolids were significantly lower than those in biosolids produced by the TH-AD process. Average concentrations of DEHP, DiNP, and DiDP in limed biosolids were 29.2, 18.7, and 7.47 ng/mg, respectively, and 66.7, 48.2, and 22.5 ng/mg, respectively, in TH-AD treated biosolids. Biosolids samples are still being processed for BBP and DEHA analysis. This study indicates that while the TH-AD is an efficient process for the reduction of wastewater solids, reduction of pathogens, and enhanced production of biogas, it may lead to an increase of plasticizer concentrations in biosolids, as is seen with DEHP, DiNP, and DiDP.

WE094
Transport mechanisms of biocides in renders
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Biocides are present in many building materials to protect them against microbial growth. One such material is render where silicone or acrylate is used as binder. After application, the biocides in the render are slowly conveyed to its surface where they are washed off during rain. While this has been shown in a number of studies, there still is little knowledge on how the biocides are transported within the render. In this study, we investigate the transport inside the render by subjecting

render tiles to various wetting and drying conditions, after which we slice them in steps of 300 μm. The biocide concentration in each slice is determined and biocide profiles from the substrate to the render surface determined. The biocides are isoproturon, octyl-isothiazolinone, diuron, terbutryn, cybutryn (irgarol 1051), dichlorooctyl-isothiazolinone, iodocarb, and tebuconazole. The render tiles are made by placing approx. 5 mm of render on plastic carriers of 55x110 mm. The slicing is done with a custom made grinder with a diamond grinding head. The rotation speed of the grinding head is slow and the render tile continuously cooled with water to ensure that the render does not heat up and denaturize the biocides. The slurry from the grinder is collected and analysed for biocides. The homogeneity of the biocides in the untreated render was tested and it was found that the biocide concentration is highest at the render surface. This is most likely caused by the coarse particles (sand) of the render not being homogeneously distributed. However, the biocide distribution in the untreated render tiles is consistent and can hence be used as boundary condition for the later data interpretation. The first experiments with continuous wet render showed that 4 of the biocides leached rapidly, reaching close to zero concentration within 1 month. Other biocides leached much slower, and were still present after several months. For all biocides, a gradient from the substratum to the render surface was evident already after 2 weeks. The study indicates that the transport in the render cannot be described as simple Fick's diffusion in an inert media but must take sorption to the render material into account. Substances that sorb strongly to the render matrix will tend to be retarded in their transport through the (probably) water filled pores of the render, while substances that sorb less will tend to move through it more unhindered.

WE095

Passive sampling technique with ELISA assay detection to monitor neonicotinoid insecticides in surface water

Y. Kameda, Chiba Institute of Technology / Department of Creative Engineering

Neonicotinoid insecticides are widely used in Japan. Especially, seven neonicotinoid insecticides such as Imidacloprid, Acetamiprid, Thiocloprid, Clothianidin, Dinotefuran, Thiamethoxam and Nitenpyram are popular. Their usage began from the beginning of 1990 and is increasing till 2008 in Japan. Recent annual total usage of seven insecticides is not increasing, approximately 400 tons per year in Japan. However very little is known about their occurrence, their behaviors and their ecological risk in Japanese environment. Although their monitoring in surface water including storm water, wastewater and sewage treated water, which arevery important to Japanese aquatic environment, is necessary to their ecological risk assessment, there are several problems to be solved. One problem is their difficult analytical method by using LC/MS/MS. Moreover, LC/MS/MS is very expensive. As a result, only a few scientists can conduct monitoring and researches. The other one is their monitoring method which is constituted by only grab sampling. Grab sampling is a very fundamental and effective sampling method when the "sampling frequency" is high. However, high frequency investigations and screenings is not realistic to Japanese government and local government. In this study, a novel monitoring method to measure neonicotinoid insecticides in surface water was developed. This method is constituted of two techniques, passive sampling technique and ELISA assay detection technique. Passive sampling technique is able to concentrate pollutantsin the field, to accumulatethem on to absorbant disks during deployment and to evaluate time-weighted average concentration. ELISA assay detection is very simple, rapid, selective and sensitive analytical technique. In this study, a new monitoring and analytical method has been developed to combinepassive sampler "Chemcatcher®" and ELISA commercial kits. This presentation shows comparison between concentration data by LC/MS/MS and those by ELISA technique in grab water samples, calibration tests for sampling rates of neonicotinoid insecticides and their occurrence in surface water in Japan.

WE097

Equilibrium Sampling indicates Increased PAH Exposure during Digestion of Sewage Sludge

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Polyaromatic hydrocarbons (PAHs) is a large class of suspected carcinogenic and mutagenic substances in environmental contaminations of sediments and soils. The sources to PAHs are petrogenic, pyrogenic or biogenic. Sewage sludge is a product of waste water treatment which farmers are encouraged to use as soil fertilizer and is a potential source of PAH contamination for the soil environment. In the present study equilibrium sampling with silicone coated jars was applied to WWTP sludge before and after digestion in order to i) obtain an extract containing hydrophobic pollutants ready for injection into GCMS instruments, ii) determine the concentration in silicone ($C_{Silicone}$) at equilibrium with the sludge and iii) convert these concentrations into freely dissolved concentrations (C_{Free}) and chemical activity ratios (ARs). Secondary and digested sludge were obtained from three Danish wastewater treatment plants (WWTPs). Within 24 hrs, equilibrium sampling of sludge was initiated, adding sodium azide to prevent biological activity. Equilibrium sampling was conducted in jars coated on the inside with silicone in four thicknesses. After equilibration with sludge the silicone was extracted with 4 mL ethyl acetate which was analysed for nineteen parent and 21 alkylated PAHs on GC-EI-MS using large volume injection. Quantification was done by isotopic internal standard calibration. From the linear relationship observed when plotting mass of PAH against the mass of silicone, the concentration of PAH in the polymer ($C_{Silicone}$) was obtained**.** The chemical activity ratio (AR) was then determined as ratio of $C_{Silicone}$ between secondary and digested sludge. AR expresses the difference in thermodynamic potential of the PAHs before and after sludge digestion. The freely dissolved concentrations (C_{Free}) were calculated using silicone-water distribution coefficients ($K_{Silicone,w}$) for the specific polymer, and compared to levels in sediment from the Baltic Sea. There is a clear trend that the concentration of parent PAHs increased when going from secondary to digested sludge. This increase in chemical activity or freely dissolved concentrations could have been caused by a reduction of sorption capacity in the sludge due to for instance degradation of organic carbon. Freely dissolved concentrations in secondary and digested sludge were within the same range as Baltic Sea sediment. Data from alkylated PAH will also be presented.

WE098

Application of a novel compact autonomous sampler to monitoring of micropollutant time-weighted average concentrations in particulate phase

Y. Kameda, Chiba Institute of Technology / Department of Creative Engineering

Grab sampling is a very fundamental and effective sampling method to measure micropollutants in surface water when the "sampling frequency" is high. However, high frequency sampling is difficult in most investigations and screenings. Passive sampling techniques have great advantages to estimate time-weighted average concentration of pollutants and to their on-site accumulation during their deployment. But passive sampling techniques have also disadvantages to measuring micropollutants in particulate phase. Laborious calibration for the estimation of sampling rates of micropollutants is also needed. Recently small continuous low-level aquatic monitoring "C.L.A.M." has been developed which can perform low flow rate extraction sampling at sampling sites. C.L.A.M. is very effective tool to estimate micropollutant concentration in low turbidity surface water. However sampling flow rates of water depend on water turbidity. As the result, it is difficult to estimate accurate time-weighted average concentration of micropollutants even in dissolved form during the deployment. Therefore it is very meaningful to develop novel samplers to monitor micropollutants automatically in particulate and dissolved forms even in muddy surface water for a week. In this study, a novel autonomous sampler "GRAVE" were developed and applied to surface water monitoring. A "GRAVE" is able to accumulate particulate matters which will contain hydrophobic pollutants, microplastics, heavy metals, pathogenic germs. Prototypes of "GRAVE"were created by the 3D printer at first and then final version was completed. This presentation will show accumulation characteristics of aquatic contaminants by the "GRAVE" to evaluate their application to micropollutants monitoring in aquatic environment. The "GRAVE" is very small (handbag size) , lightweight and work by only two D size cells. It can accumulate particulate matters in surface water for a week with constant flow rate. The recovery rates of inorganic compounds and phosphate was more than 80% though the recoveries of biodegradable compounds such as in organic carbons and nitrogen were low. Therefore this new sampler is very useful to estimate TWA concentration of less biodegradable compounds in particulate phase.

WE099

The role of sampling artefact in gas-particle phase characterization of orgnophosphate esters (OPEs)

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Semi-volatile compounds (SVOCs) exist in both gas and particle phases. The phase into which they partition influences their toxicity and environmental fate. The gas-particle partitioning ratio (coefficient) is measured using an active air sampler consisting of filters and sorbents. Concentrations measured on filters and sorbents are defined as particle and gas phase fractions, respectively. This operational definition is reliable in the absence of sampling artefacts such as breakthrough losses and gas-phase absorption to filter. Organophosphate esters (OPEs), as SVOCs, have been classified as particle-phase compounds because many studies have reported 100% capture on glass fibre filters (GFFs). However, all OPEs are unlikely to be exclusively particle phase since they have vapour pressures ranging from 10^{-2} to 10^{-5} Pa for TCEP to TBOEP, respectively. The Junge-Pankow model predicts that some OPEs should be in the gas-phase while others should be in the particle phase in ambient indoor and outdoor air (Sühring et al. 2016). We hypothesize that some OPEs are in fact found in the gas phase but are mischaracterized as being in the particle phase due to measurement artefacts due to electron donor-acceptor interactions operating between the film of water covering the GFF, the OPEs and the GFF, which are all polar in nature. To investigate the artefact or "pseudo-particle phase behaviour", we predicted the sorptive capacity of GFF for selected OPEs and brominated flame retardants (BFRs) as a function of relative humidity (RH) using the poly-parameter linear free energy relationship (pp-LFER) model of Goss and Schwarzenbach (2002). The volume of air that GFF can sample before compound breakthrough (V_B) was used as a surrogate for the sorptive capacity of GFF for that compound. $V_B$values of OPEs at RH of 45% ranged from 10^3 to 10^{10} m^3 for TCIPP to TBOEP, except for TCEP which had a low value of 10 m^3. $V_B$values for OPEs were at least three orders of magnitude higher than those of BFRs of comparable or lower vapour pressures that are reported to be in the gas phase. They were also higher than ~ 170 m^3 of air typically sampled using active air samplers. The pp-LFER model results indicate that a hydrated GFF can retain the total amount of OPEs contained in air during typical active sampling regimes, in the absence of particles. Future experimental work should study sorption to GFF under conditions of controlled relative humidity.

WE100

Optimisation of the experimental conduct to accurately determined Kd / KF values of low adsorbing compounds

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The determination of accurate adsorption parameters is a critical key parameter for the assessment of the leaching properties of low adsorbing compound's through the vertical profile of soils. In case of expected low adsorption of a chemical compound, an initial soil to liquid ratio of as low as 1:1 is generally chosen to increase the amount adsorbed onto the soil phase ideally to amounts > 20 %. However, after phase separation using classical centrifugation techniques, the amount of residual liquid phase entrained in the soil pellet can still be so high, that the analytical error is so substantial that no meaningful K-values and no reliable adsorption isotherms can be determined even if the direct method (analyzing both phases – soil phase by solvent extraction) was used. The pertinent guideline on adsorption (OECD 106) refers to a fundamental peer reviewed paper (*Reference 61: Boesten (1990) "Influence of soil/liquid ratio on the experimental error of sorption coefficients in pesticide/soil systems". Pest. Sci. 30: 31-41*). In this paper, it is stated in chapter 3.2, that accurate determination of K value is possible, if the product with the soil/solution ratio (P value) is > 0.3. Additionally Boesten 1990 concludes, that removing the liquid phase before soil extraction as much as possible by centrifugation over a filter results in a soil/solution ratio of at least 3 kg/L. Then the lowest K-value, which theoretically can be measured with sufficient accuracy is as low as 0.1 L/kg. We present here an optimised experimental approach on how the amount of liquid phase in the soil after phase separation can be reduced to such an extent that a solid/solution ratio before soil extraction of greater than 3 kg/L is obtained after centrifugation over a filter. As a result, K-values can thus be accurately determined at values lower than 0.1 L/kg. A comparison of the K-values obtained by the classical approach, the attempt to start with a higher initial soil/solution ratio and the modified new experimental approach are presented. In the rare cases where even an advanced centrifugation/filtration technique does not lead to a sufficient high soil/solution ratio to exceed P-values of 0.3, a statistical approach is presented how the reliability of the isotherm determination can furthermore be evaluated.

WE101

Proposed biocatalytic treatment for degradation of hormones and antibiotics as emerging contaminants

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Since the recent detection of pharmaceutical compounds (PhC) in water bodies these compounds are considered as emerging pollutants. Hormones and antibiotics are of particular concern as pollutants due to their proven toxicity and environmental impact. The negative effects of these PhC on aquatic life and human health argue the need for effective evaluation and optimization for removing those compounds applying preferentially green techniques. Environmental biocatalysis is one of such techniques, which is a friendly environmental process. The removal of PhC has been proven to be effective and fast degradation has been reported. In addition, reaction products have showed lower toxicity and higher degradability. In the present study the oxidative capacity of manganese peroxidase (MnP) was tested against three hormones (estrone -E1-, 17b-estradiol -E2- 17a-ethinylestradiol -EE2-) and two antibiotics (sulfametoxazol and sulfadiazine). Optimal removal conditions were determined by applying a Central Composite Design constructed from a 2^3 producing 20 experiments by adding axial points and tested for their analysis by response surface methodology. The independent variables were enzyme amount, hydrogen peroxide concentration and reaction time, at two levels (0.10-1.06 U, 0.33 to 1 mM and 5-15 min., respectively), meanwhile the dependent variable was %removal. The next optimal conditions for MnP catalysis were determined: 0.49 mM hydrogen peroxide, 086 IU and 5 minutes of reaction time. Under these conditions, 91, 89 and 98% removal of EE2, E2 and E1 was observed, and 80% and 100% for sulfametoxazole and sulfaziadine were attained respectively. Finally, the first-order reaction constants were calculated at the optimal conditions, with an average value of 0.92 min^{-1} for hormones, and for antibiotics 0.23 min^{-1}. The biocatalytic transformation of hormones and sulfamide antibiotics is envisaged as a potential technology for polishing steps at wastewater treatment facilities.

WE102

Study of the Degradation of Bisphenol A (BPA) by basidiomycetes via HPLC in urban reservoir waters

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Contamination of water resources is one of the major environmental problems of our time, and the concern micropollutants - substances that appear in concentrations in order of μgL^{-1} and ngL^{-1} has increased significantly in recent years Among the substances which can be classified as micropollutants include the compounds with estrogenic activity such as of bisphenol A (BPA). Endocrine disruptors with estrogenic activity are those that arouse most concern to health workers, as they are extremely biologically active and have a solid introduction into the environment. The removal of emerging pollutants through stabilization ponds - the leading technology in wastewater treatment in Brazil - shows variation in efficiency. Other processes for removing pollutants are emerging reverse osmosis, chemical oxidation, ozonolysis, and the other tooth. Procedures are expensive; they require many modifications to existing processing lines and therefore large financial investment, fact that leads to the impossibility installation thereof. However, the oxidation of pollutants arising by enzymes derived from microorganisms has been considered a novel and potentially effective technology for the removal of these substances in wastewater that will eventually be discharged into the environment. In our laboratory we are studying the degradation of BPA using Trametes and Pleurotus basidiomycete fungi using 250µL of BPA solution with concentration of 1mg / L in the erlemeyer content of the culture media with the fungi prepared according to protocols standardized with 3 replicates of each medium Of culture under study for each fungus as well as biotic and abiotic control followed the degradation for 7 days and evaluated degradation via Agilent 1220 Infinity HPLC-DAD using mobile phase 50% H2O: ACN pH 3.0 adjusted with phosphate buffer, 40 ° C , Injection of 10µL in 280nm and we performed collections in the 0, 72hs, 144h times in these periods in intervals of 0, 30, 60 and 120min. It was observed that at time 0 there was already an immediate reduction of 10% of the initial concentration of BFA and in 72 and 144 hours a reduction of 75% of the initial concentration of BPA at the moment we are adjusting the methodology and to validate the method for application in samples of the Billings Dam And evaluating the concentration of lignolytic enzymes that act in the process.

WE103

Sorption of nitro explosives to polymer/biomass-derived biochar: Affecting factors and toxicity

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Factors affecting sorptive removal of nitro explosives [2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)] to polymer/biomass-derived biochar were investigated through batch experiments. Compared to Rice straw (RS)-derived biochar, the sorption of nitro explosives to polymer/RS-derived biochar was greatly enhanced. The type and amount of polymer did not significantly affect the sorption of nitro explosives to polymer/RS-derived biochar. Due to increasing aromaticity of biochar and decreasing carbon residues from polymer, the effect of pyrolysis temperature at elevated temperature was not marked. Surface treatment with acid or oxidant did not change the sorption capacity. Possible polymer residues were identified via GC-MS analysis. Toxicity characteristic leaching procedure (TCLP) and Microtox® bioassay analysis indicated that polymer/RS-derived biochar did not show possible harmful effects. Our results suggest that polymer/RS-derived biochar can be effectively and safely used as a sorbent to remove nitro explosives in natural environment and engineered systems.

WE104

Removal of recalcitrant hydrocarbons present in Diesel-contaminated soil by serial processes of oxidation and biodegradation

Y. Park, S. Lee, R. Bajagain, Kunsan National University; S. Jeong, Kunsan National University / Department of Environmental Engineering

In the modern society, the use of fuel oil is increasing for industrial and our living purposes. As the fuel use increases, spillage and leakage of oil have been also increasing and resulting in soil and groundwater contamination. Fuel oils present in soil may be potential and actual sources that may result in harmful health and ecological effects. Fuel oils are a complex mixture of hydrocarbons. Low-molecular hydrocarbons are readily biodegraded in the natural soil system. However, some recalcitrant hydrocarbons tend to persist for a long time in the environment. Diesel includes recalcitrant hydrocarbons to the natural degradation and may act as potential and actual sources of harmful human and ecological effects. Landfarming is a widely used bioremediation technology for fuel oil-contaminated soils. Although soils contaminated by gasoline are readily remedied by landfarming, Diesel contaminated soils are not well remediated by biodegradation. The purposes of the study were to find the fraction of hydrocarbons that may be recalcitrant to biodegradation and achieve enhanced removal of recalcitrant hydrocarbons by using oxidation-biodegradation. This study showed that recalcitrant hydrocarbons to biodegradation were C_{18}~C_{22} present in Diesel. This study used two different oxidants for pretreatment prior to application of biodegradation. Hydrogen peroxide and sodium persulfate were used for pre-treatment of Diesel contaminated soil. This study also developed oil-degrading microbes (K-6) from C_{18}~C_{22} accumulated biological cultures. Pretreatment of Diesel contaminated soil by using oxidants reduced recalcitrant hydrocarbons and facilitated biodegradation. (*This work was supported by National Research Foundation of Korea* (NRF-2015R1D1A1A01059664)).

WE105
SETAC Chemistry Interest Group
A.J. Jones, DuPont Crop Protection / Institute of Environmental Toxicology

Advances in Soil Ecotoxicological Risk Assessment of Chemical Stressors (P)

WE106
Ecotoxicological assessment of wastewater soil (sludge) on survival and reproduction of Enchytraeus albidus (Oligochaeta) in the Maluti-A-Phofung municipality
P.M. Mosolloane, University of the Free State / Zoology and Entomology; P. Voua Otomo, University of the Free State / Department of Zoology and Entomology; E. Bredenhand, University of the Free State / Zoology and Entomology
Sewage sludge is one of the by-products of wastewater treatment. The aim of this study was to determine the effects of sludge on survival and reproduction of the oligochaete species *Enchytraeus albidus*. this was done to assess the potential harmful effects of improper sewage sludge disposal on soil invertebrates. Sludge was obtained from a wastewater treatment plant in Phuthaditjhaba and in Harrismith. Ten adult specimens of *E. albidus* were exposed to sludge mixed with clean Organisation for Economic Cooperation and Development artificial soil. Sludge percentages in the mixed treatments ranged from 0, 25, 50, 75% and 100%. The specimen were exposed for 21 days at 20°C. Thereafter, the number of surviving adults and juveniles produced were counted. For Phuthaditjhaba, there was no significant effect of sludge on the survival of *E. albidus*. A LC_{50} could not be calculated but a LC_{20} (47.90% sludge) was calculated. Consequently, an EC_{50}= 58.53% sludge was calculated for reproduction. For Harrismith, similarly, significant effects were only found on reproduction in the 75 and 100% sludge treatment ($p< 0.01$). A LC_{50} could not be calculated but an LC_{20} (56.25% sludge) was calculated. The EC_{50} for reproduction was 43.61% sludge. These results indicate that the sludge produced at the Wastewater Treatment Plant in Phuthaditjhaba and Harrismith has the potential to cause harmful effects on soil dwelling organisms such as oligochates.

WE107
Biochar amendment in dimethoate contaminated soils: toxicity assessment using the collembolan Folsomia candida and the dicotyledonous plant Brassica rapa
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Soil contamination is a worldwide problem urging for resolution. Several techniques are being developed and upgraded to see their efficacy in removing organic and inorganic contaminants from soils. Biochar is a carbonaceous material that, aside from being a soil amendment, can immobilize chemical compounds due to a large and reactive specific surface area, potentially turning them unavailable for the soil biota. Therefore, the aim of our study was to test the biochar's capacity to immobilize dimethoate in agricultural soils and, therefore, decrease the toxicity to soil organisms. To test this hypothesis, two biochar rates – 2.5% and 5% (w/w) - and two standardized organisms - the collembolan *Folsomia candida* and the plant *Brassica rapa* - were chosen to assess pesticide immobilization by biochar by evaluating changes in dimethoate toxicity upon soil amendment. As a complement, chemical analyses were also performed on the soil and on the soil's pore water to check if the chemical concentration decreased. Our results showed that dimethoate may sorb onto biochar by looking at dimethoate concentration in the pore water, thus proving that it can influence the bioavailability and the toxicity to these organisms. For the reproduction test with collembolans, the offspring production and the survival rate were affected positively with the biochar treatment, independent of the percentage of biochar in soil amendment. For the germination test, endpoints such as length and fresh weight of the aerial part of the plants were also affected positively with biochar addition; however, biochar's influence was less efficient because there was still a dose-response curve for dimethoate observed. With our results, we conclude that biochar can alleviate dimethoate pollution, by decreasing the bioavailability to soil fauna and flora.

WE108
Leaching of weathered polychlorinated biphenyls (PCBs) obtained from a contaminated site: role of dissolved organic carbon and saturation conditions in a soil column experiment
C. Vitale, University of Insubria; D. Zati, University of Milan; A. Di Guardo, University of Insubria / Department of Science and High Technology
Exposure assessment in contaminated soils is usually performed by the evaluation of the total residual concentrations of target pollutants in soils (e.g. ∑PCBs). This approach does not take into account the actual mobility and bioavailability of the contaminants and may be inappropriate especially for historically contaminated soils where sorption processes may be scarcely reversible due to the presence of bound/weathered residues. Therefore, assuming that equilibrium conditions would occur using standard partitioning coefficients (e.g. Koc/Kd) may lead to misleading results in modelling attempts. Therefore, the estimation of the contaminant fraction available for leaching and transport in different soil conditions is recommended. During the last decades, several authors studied the release of pollutants from soil by using soil column leaching tests but the laboratory experiments were often performed in scarcely realistic conditions (e.g. fresh spiked contaminants). Additionally, often some variables influencing the mobility of contaminants were neglected, as well as the statistical scheme was poor (e.g. lack of replicates). In this context, a soil column leaching experiment was performed to evaluate: 1) the effects of dissolved organic carbon (DOC) content in the leaching solutions, 2) equilibration time, 3) soil saturation conditions on leaching fluxes of selected weathered PCBs (PCB 28, 52, 101, 138, 153, 180, 209) present in an historically contaminated soil. These effects were evaluated collecting leached samples at different contact time (2, 5, 7, 48 days), in flow vs. no flow conditions and in saturated vs. field capacity conditions. The results show that the most influential factors are the DOC content in non-equilibrium conditions (flow condition samples) and the soil saturation conditions.

WE109
Soil risk assessment for glyphosate and AMPA
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The herbicidal active substance glyphosate is a broad-spectrum systemic herbicide used widely in agriculture, horticulture, private gardens and in public infrastructure, where it is applied to areas such as road sides, railway tracks and parks to control the growth of weeds. The exposure risk from glyphosate and the primary soil metabolite aminomethylphosphonic acid (AMPA) on representative species of soil structuring earthworms (*Eisenia fetida*), re-cycling springtails (*Folsomia candida*), beneficial predatory soil mites (*Hypoaspis aculeifer*) and effects on nitrogen-transformation processes by nutrient cycling soil micro-organisms were assessed under controlled laboratory conditions based on internationally recognized testing guidelines (OECD). For earthworms, the reproductive no observed effect concentration (NOEC) was > 473 mg acid equivalent (a.e.)/kg dry soil for glyphosate, and 198.1 mg/kg dry soil for AMPA. For predatory mites, the reproductive NOEC was 472.8 mg a.e./kg dry soil for glyphosate and 320 mg/kg dry soil for AMPA. For springtails, the reproductive NOEC was 587 mg a.e./kg dry soil for glyphosate and 315 mg/kg dry soil for AMPA. Soil nitrogen transformation processes were unaffected by glyphosate and AMPA at 33.1 mg a.e./kg soil and 160 mg/kg soil, respectively. Comparison of the achieved soil organism endpoints with worst-case soil concentrations (Max PEC_{soil}) expected for glyphosate (6.62 mg a.e./kg dry wt soil) and AMPA (6.18 mg a.e./kg dry wt soil) for a bare soil application of a glyphosate containing formulation, indicate that the risk to soil invertebrates and microorganisms from glyphosate and AMPA is acceptable with exposure safety margins of between 5 and 88.

WE110
Statistics in Earthworm Field Testing
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Earthworm field tests are carried out as part of the risk assessment of plant protection products according to the ISO guideline 11268-3 (ISO 2014) and considering De Jong et al., 2006. These documents provide information on experimental design, test site requirements, data assessment and validity criteria of the test, but do not give clear and up-to-date guidance on the required statistics. Within the Draft Scientific Opinion on risk assessment of plant protection products for in-soil organisms (EFSA 2015) the minimum detectable difference (MDD) concept to evaluate the statistical power of the test system is mentioned. In addition, new approaches for statistical analysis of (earthworm) field tests are available. Exemplarily, typical datasets from a set of 40 earthworm field studies will be analyzed using different methodologies (parametric and nonparametric standard procedures, as well as more complex multivariate statistical methods, i.e. also considering the response of the whole community or considering a possible correlation). Key statistical parameters of the whole dataset are also presented (e.g. MDDs, spatial and temporal variation of the data). The discussed field studies were carried out in the years 2003 to 2016 on arable soil and grassland in Germany, France, and Spain. Key aspects and limitations of the different approaches will be discussed and recommendations given. **References\n** De Jong, FMW., Van Beelen,

P., Smit, CE. & Montforts, MHMM., 2006: Guidance for summarising earthworm field studies – A guidance document of the Dutch platform for the assessment of higher tier studies. RIVM, The Netherlands, 47 pp. EFSA, 2013: Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Panel on Plant Protection Products and their Residues (PPR). Parma, Italy. EFSA J. 11(7):3290. EFSA, 2015: Draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Panel on Plant Protection Products and their Residues (PPR). EFSA-Q-2011-00978, Parma, Italy. ISO, 2014: ISO Guideline 11268-3: Soil quality – effects of pollutants on earthworms. Guidance on the determination of effects in field situations.

WE111
Stratified activity based sampling of soil micro-arthropods
F.M. Bakker, Eurofins-Mitox; S.B. Dehelean, Mitox Trial Management BV; R. Delhem, F. Faraji, A. Grove, J. Roig, EurofinsMitox Consultants
Soil micro-arthropods such as mites and springtails exhibit specific vertical distributions and spatial dynamics. This has clear implications for the degree of exposure to toxicants they experience, but also for the manner in which exposure translates into experimental measures of effect. For species with a static distribution, these effects are clearly linked to the stratum of preference in relation to soil mobility of the toxicant. For species with vertical mobility patterns the relationship between exposure and effect may be complex because whereas certain strata may act as a refuge, others will be a trap. In combination with autecological characteristics this may give rise to source-sink dynamics, the outcomes of which on overall effect values are unpredictable. To understand the complex behavior of such systems appropriate sampling is crucial. In this context an adequate sampling technique should enable the sampling of specific soil strata Furthering the idea of Dehelean et al. (2015), we developed an activity based trapping method that enables stratified field sampling such that each trap can sample user defined strata of up to 3 cm soil depth intervals, with multiple levels being sampled by the same trap. In a field study design that was comprised of 4 traps, with 4 strata per trap (0, 10, 20 and 25 cm) for each of 12 concentrations of chlorpyrifos and 8 traps for a water control, we tested the responses of different taxa of collembola and mites at different soil layers. In a subsequent sample we focused on 0, 2, 5 and 10 cm depth At the moment of writing not all material has been taxonomically identified, but the first results show that different collembola taxa exhibit different vertical distributions/activity patterns and different sensitivities. For example *Sminthurinus elegans* (Katiannidae) was found almost exclusively at the soil surface and had a clear dose-related effect profile at 0 cm. *Sphaeridia pumilis* (Sminthurididae) was found at the soil surface and to a lesser extent also at 0-10 cm and displayed a dose-related response at both these depths. For Entomobryidae (predominantly *Lepidocyrtus lanuginosus*) on the other hand individuals were trapped at all sampling depths, but a monotonous effect increase was only observed at the surface. The curve was however more flat, which showed higher variability. At the conference we will present a comprehensive overview of effects on mites and collembola at different depths, including 0-2 and 2-5 cm

WE112
General framework for assessing the risks for in-soil organisms exposed to Plant Protection Products (PPP)
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The Panel on Plant Protection Products and their Residues (PPR Panel) at the European Food Safety Authority (EFSA) and its Working Group on “Non Target Arthropods and In-soil organisms” has published a Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms, which was open for public consultation. The main scope of the work was to take into consideration: (i) the entry into force of the Regulation 1107/2009 replacing the Directive 91/414/EEC; (ii) the need for developing specific protection goals and (iii) the inclusion of new scientific elements in the environmental risk assessment of in-soil organisms. The present contribution focuses on the general framework elaborated in the Scientific Opinion on how the risk for in-soil organisms should be addressed in an improved assessment strategy. The group reviewed available knowledge on ecology, ecotoxicology and exposure of in-soil organisms in agricultural landscapes, focusing on species (group) traits determining the organisms’ vulnerability to intended uses of PPP. In order to provide an appropriate prospective risk assessment for in-soil organisms following a tiered approach, it was important to identify different sources of uncertainties in the extrapolation from single species tests to responses of in-soil organisms’ communities after PPP intended uses. It was concluded that, for the time being, different sources of uncertainties need to be addressed specifically in an amended risk assessment scheme. The proposed scheme has two components: (i) assessment of effects in laboratory, field or semi-field studies; (ii) assessment of long-term effects using population modelling. The first component, which has the goal of assessing the effects of PPP on in-soil organisms’ communities, addresses possible direct and indirect effects of intended PPP uses. The second component addresses the effect of year on year application of PPPs with appropriate population models. In order to link exposure assessment and ecotoxicological responses of in-soil organisms, the Panel and its Working Group has reviewed the available knowledge on temporal and spatial profiles of PPP active substance in soils after application and the distribution and movements of different organisms groups in the soil profile. The conclusions on how to link best exposure and effect assessment are presented together with the specific requirements for different soil organisms’ groups.

WE113
Species sensitivity distributions for soil organisms: experiences for metals
K. Oorts, ARCHE; I. Schoeters, Rio Tinto
The species sensitivity distribution (SSD) approach is generally acknowledged as a useful tool in tiered risk assessment, but opinions vary on how the data should be considered for soil organisms. In contrast to the aquatic compartment, no detailed guidance is available on the use of the SSD approach for soil organisms and a clear need for guidance development was identified at the Topical Scientific Workshop on Soil Risk Assessment co-organized by EFSA and ECHA in October 2015. Discussion points are e.g. the taxonomic groups to be minimally represented in the SSD and the combination of microbial mediated processes and single species tests into one SSD. During the last 10 years, the SSD approach has been used for several data-rich metals and metalloids (e.g. Ag, B, Cd, Co, Cu, Mo Ni, Pb, V, Zn) for derivation of PNEC values under the REACH regulation. Based on this experience, industry guidance has been formulated for the minimum data requirements (Metals Environmental Risk Assessment Guidance (MERAG), http://hub.icmm.com/document/255). Some of these metals also have applications as biocides or pesticides. Because protection targets and the risk assessment approach can differ especially between the pesticides and REACH regulations, the use of the SSD approach also may differ across these regulations. The information collected for these metals is used to illustrate the impact of individual taxonomic groups on the HC5 or PNEC and the implications of combining all data into one SSD versus splitting species and functions. Normalization models to account for the effect of varying soil properties on toxicity of metals to soil organisms allow to correct for this source of variation and to avoid additional uncertainty on intra- or inter-species variability leading to a more focused discussion of the impact of specific organisms or trophic levels. The results show that there is no species or group (plants, soil invertebrates, or microorganisms) that is consistently most or least sensitive. The microbial endpoints are generally distributed across the range of the combined SSD and inclusion of the microbial data decreased the uncertainty for the estimated HC5 or HC50 values (i.e., smaller confidence intervals), thereby increasing the robustness of the SSD. This poster will present the learning lessons from the experience with metal SSDs for soil organisms.

WE114
Effects of Bacillus cereus inoculum on the ecotoxicity of metal-based fungicides towards Eisenia andrei
M. Maboeta, North-West University / Unit for Environmental Sciences and Management; O.G. Oladipo, North-West University / Unit for Environmental Sciences & Management; A. Burt, North-West University
Earthworms are vital in soils and their use in soil ecotoxicological studies and soil quality assessment is well documented. Copper oxychloride (60% copper) and mancozeb (16% manganese and 2% zinc) are fungicides utilised in orchards and vineyards. We investigated the effect of *Bacillus cereus* inoculum on the ecotoxicity of these metal-based fungicides towards *Eisenia andrei* in spiked soils. A 24 hours’ broth culture of bacterial strain; *Bacillus cereus* isolated from mining sites (shown tolerance to 600, 600 and 200 ppm Mn, Zn and Cu respectively in previous studies) was introduced into the spiked soils. Experimental trials included avoidance-behavior, growth and reproduction following standardized protocols (OECD and ISO). Mature clitellate earthworms were exposed in inoculated and non-inoculated substrates of mancozeb (8, 44, 800 and 1250 mg/kg), copper oxychloride (200, 450, 675 and 1000 mg/kg) and controls. Avoidance-behavior results revealed that *Eisenia andrei* demonstrated preference for inoculated substrates. Biomass results indicated that, while the non-inoculated test substrates showed significant ($p < 0.05$) difference to the control, inoculated-test substrates showed no significant ($p > 0.05$) difference. Significant mortalities were only observed in the highest exposure concentrations for both fungicides. At the two lowest fungicide concentrations, inoculated test soils had 75 and 85% increase reproductive success respectively compared to the non-inoculated substrates. However, at higher mancozeb (800 and 1250 mg/kg) and copper oxychloride (675 and 1000 mg/kg) concentrations, reproductive success in both test soils was negatively ($p < 0.05$) affected. Although, Cu, Mn and Zn soil and earthworm tissue contents showed significant ($p < 0.05$) difference to the control in both test soils, earthworm bio-concentration factors were < 1 except for Zn with 1.28 to 2.57 in inoculated and 1.22 to 3.72 in non-inoculated soils. It can be concluded that

Bacillus cereus decreased the ecotoxicity of metal-based fungicides towards *Eisenia andrei* by enhancing preference, biomass, survival and reproductive success in metal-based fungicide soils especially at low concentrations. *Bacillus cereus* can therefore be explored as an effective bio-augmentation tool for bioremediation of metal contaminated soils.

WE115

Placing arbuscular mycorrhizal fungi on the risk assessment test batery of plant protection products

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Arbuscular mycorrhizal fungi (AMF) can establish symbiosis with 80% of different plant families contributing to crop productivity and ecosystem sustainability. Thanks to their wide distribution, they are able to provide their crucial services to almost all terrestrial ecosystems, including agroecosystems.In the process of development of an opinion on the science behind the risk assessment for in-soil organisms, the EFSA Panel on Plant Protection Products and their Residues (PPR) identified AMF as key drivers of several ecosystem services potentially impacted by PPPs, particularly nutrient cycling and soil formation. AMF have been shown to enhance plant productivity by improving nutrient and water uptake by plants, the later giving them competitive advantage towards extreme climatic events, especially related to water stress. They play a crucial role in the formation and maintenance of soil aggregates. Through their extensive network of intra- and extraradicular mycelium, their ability to render surfaces hydrophobic and the production of glomalin, AMF are important in maintaining stable soil aggregates. Specific protection goals have been proposed by EFSA PPR Panel for AMF aiming at protecting the functional group from unacceptable effects when exposed to intended uses of PPP. After reviewing the current risk assessment scheme for in-soil organisms and based on the fundamental role AMF play in agricultural areas, the EFSA PPR Panel recommends to routinely test AMF in the context of PPP authorization, since AMF are not covered by the standard nitrogen transformation test. An ISO protocol with *Funneliformis mosseae* (formerly *Glomus mosseae*) is already available. However, further adaptation of the endpoints measured or its extension to other species/strains is recommended. Results of current developments on AMF testing are presented. Focus is given to further development of ISO 10832:2009 protocol, in particular (i) the use of other AMF species, (ii) to assess the sensitivity of other parameters of the asymbiotic phase, and (iii) test performance under different temperatures and soil types. Results from tests performed in vitro with modified roots to assess effects and sensitivity of parameters from the symbiotic phase will also be presented.The work presented aims to stimulate the discussion on the feasibility and further developments to make this protocol employable on a routine basis in the in-soil risk assessment of plant protection products in the future.

WE116

Microbial respiration of added 13C-labelled sugar in the field - A step towards a non-invasive assessment of ecological functions of polluted sites of high ecological value

I. Rijk, MTM Research Centre, Orebro University / Ecosystem Ecology; A. Ekblad, MTM Research centre Örebro University / Ecosystem Ecology

Ecological risk assessment of polluted areas is slowly shifting from a chemical-value based approach to a more holistic view on the performance of critical soil functions and ecosystem services. Microorganisms have an essential role in soil nutrient cycles, and may be studied to identify whether critical soil functions (e.g. carbon and nitrogen cycles) are impaired. Although most methods to study microbial functioning are laboratory based, field methods are preferred because digging, storage and sieving of soils alters both the mobility of pollutants and soil biology. To assess whether the microbial activity was disturbed in a Pb-polluted NATURA2000 area, an exploratory field experiment was carried out using a non-invasive technique to study soil microbial C-mineralization *in situ*. We measured soil respiration rates and isotopic signatures of respired CO_2 one hour and two days after inducing polluted (mean=2300 mg Pb/kg soil) and non-polluted (< 50 mg Pb/kg soil) plots with cane sugar (naturally ^{13}C-labelled) and water (control). In this way we could distinguish between respiration of endogenous C (root and microbial) and of the added C (microbial). Preliminary results show that microorganisms respire the added sugar at similar rates in both unpolluted and polluted soils. The results suggest that the Pb was not affecting the microbial activity (one hour measurement) nor the growth of the microorganisms (two days). This approach is a first a step to a more functional ecological risk assessment directly in the field. This method could be especially beneficial to polluted areas with a well-developed ecosystem and a high ecological value (e.g. NATURA2000), where the ecological status of the site is crucial to determine whether remedial actions are needed to be taken.

WE117

Impact of pesticides on non-target fungi in soils and sediments

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In this study, we refer to 'non-target fungi' (NTF) in soils and sediments and focus on the arbuscular mycorrhizal fungi (AMF). Our aim is to present the AMF to a wide audience interested in risk assessment of fungi in soil and sediments, and to summarize the historical knowledge on AMF/pesticide/pollutant interactions (e.g. Sieverding & Leihner 1984, Trappe et al. 1984). AM fungi play important roles in the rhizosphere of virtually all terrestrial and aquatic ecosystems colonised by vascular plants and fulfil multiple functions in their soil environments. Furthermore, they can easily be propagated in terrestrial micro- and meso-cosms, and recently they were successfully cultured even on plants in aquatic meso-cosms. They form rather large spores and high amounts of easily detectible hyphal mycelia and intraradical vesicles and arbuscles, and thus can be easily detected also by promising molecular detection tools. To our opinion, AMF represent a genetically rather small, but omnipresent functional fungal group, for which specific protection goals and risk assessment procedures should be established. EFSA PPR Panel (2016) suggested to follow a spore germination test on the AM fungus *Funneliformis mosseae* (synonym: *Glomus mosseae*) referring to ISO 10832:2009. In this protocol also an AMF hyphal root colonisation test was proposed, but the 'hyphal colonisation method' was still under investigation. Such germination, growth and reproduction impacts should be studied not only for *F. mosseae*, but also for other, possibly more sensitive species, different AM fungal groups and on the population/community level, especially for polluted soils and sediments in terrestrial and aquatic ecosystems. In conclusion, AM fungi are ubiquitous in-soil fungi and play a major role for ecosystem functioning. Their sensitivity towards pesticides, the goals for their protection in different agricultural soils and a suitable risk assessment procedure should be investigated further. References: EFSA PPR Panel 2016. Draft scientific opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms.https://www.efsa.europa.eu/sites/default/files/consultation/160503.pdf Sieverding E, Leihner DE. 1984 Effect of herbicides on population dynamics of VA mycorrhiza with cassava. J Appl Bot 58: 283-294. Trappe JM, Molina R, Castellano M. 1984. Reactions of mycorrhizal fungi and mycorrhiza formation to pesticides, Ann. Rev. Phytopath 22: 331-335.

WE118

The effects of neonicotinoid pesticides on non-target soil invertebrates in soil

J. Princz, Environment Canada / Biological Assessment and Standarization Section; E. Ritchie, Environment and Climate Change Canada / Biological Assessment and Standarization Section; R.P. Scroggins, Environment Canada / Biological Methods

Neonicotinoids are used intensively in Canadian agriculture, but only a small number of studies have assessed the risk of these substances to soil-dwelling organisms, despite the growing evidence of persistence in surface soil. Very few studies have evaluated non-target soil invertebrate species, and they are often limited to the use of artificial substrates, rather than natural soil. As a result, research was performed to evaluate the toxicity of selected neonicotinoids (thiamethoxam and clothianidin) to earthworms (*Eisenia andrei*), springtails (*Folsomia candida*) and oribatid mites (*Oppia nitens*) in a field-collected sandy loam soil; the bioaccumulation potential within earthworms was also evaluated. Test soils were amended with formulated neonicotinoid products, and effects on soil invertebrate lethality and reproduction were assessed. An evaluation of thiamethoxam resulted in effects on *E. andrei* reproduction at < 3 mg/kg, and significant effects on *F. candida* survival and reproduction at < 0.5 mg/kg dry soil; effects on *O. nitens* were only observed at > 100 mg/kg. Thiamethoxam and clothianidin results will be compared in order to identify potential trends in organism susceptibility, as clothianidin is a known degradation product of thiamethoxam. The results of this research will provide base-line toxicity and bioaccumulation data for inclusion in food web transfer modelling studies, in addition to environmental risk assessment processes.

WE119

Validation of a New Ecotoxicity Test Method for Assessing the Effect of Contaminants in Soil using Orbatid Mites

J. Princz, Environment Canada / Biological Assessment and Standarization Section; H. Lemieux, P. Boyd, Environment and Climate Change Canada / Biological Assessment and Standarization Section; R.P. Scroggins, Environment Canada / Biological Methods

Oribatid mites are currently under-represented in soil ecotoxicity testing, but are an important taxonomic group found in abundance within surface soils. Oribatids contribute to a healthy ecosystem by assisting with nutrient cycling through litter breakdown and to soil formation processes. Environment and Climate Change Canada (ECCC) method development and validation efforts have led to a standardized ecotoxicity test method using the species, *Oppia nitens* (C.L. Koch, 1839). The new ECCC test method involves a 28-d soil exposure to capture effects on adult lethality and reproduction (measured as the number of live juveniles produced). Validation of the new test method is currently under way through inter-laboratory testing involving nine participating laboratories from Canada and Europe. There are three rounds of round robin testing which include a performance test in two field soils and an artificial soil (Phase 1), a reference toxicant test using boric acid in a field soil (Phase 2), and a full survival and reproduction test involving a pesticide and standard natural soil such as Lufa 2.2 (Phase 3). The results from completed phases to date (Phase 1 and Phase 2) will be presented, along with an analysis of test variability, and performance across different soil types. The new test method is the first standardized effort for oribatid mites; the method will complement an existing suite of soil test methods (e.g., earthworms and collembolan), with applicability to both forest and agricultural soils.

WE120

Impact of three neonicotinoids on the collembolan Folsomia fimetaria, in agricultural soils

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Neonicotinoids are the most widely used class of insecticides in the world. They are applied in a prophylactic way, providing a systemic and long-term crop protection against sucking pests. In agriculture they are frequently used as seed coating for many crops and can accumulate and persist in the soil, leading to a potential risk for a wide range of non-target organisms. The effect of neonicotinoids on the reproduction of the Collembola *Folsomia fimetaria* in agricultural soils was assessed using ecotoxicological bioassays. Neonicotinoid toxicity was first evaluated for field concentrations by testing soil samples originating from Swiss agricultural crops (conventional and IP-Suisse). Their borders or ecological compensation areas were used to assess off crop exposure. Secondly, effect concentrations for imidacloprid (IMD), clothianidin (CLO) and thiamethoxam (THX) were determined using spiked natural LUFA 2.2 standard soil. A mixture of IMD and CLO was also evaluated. A chemical analysis was performed and physico-chemical parameters were assessed for field and spiked samples. Finally, a first environmental risk assessment was done, based on the measured field concentrations. IMD and CLO were detected in all field samples (with maximal concentrations of 0.023 and 0.016 mg a.i./kg d.w. of soil for IMD and CLO, respectively), supporting the hypothesis of persistence and/or dispersion. Field samples were not toxic to *F. fimetaria*. The bioassays with spiked soil samples gave EC10s of 0.109, 0.115 and 0.166 mg a.i./kg d.w. for IMD, CLO and THX, respectively, showing generally a higher toxicity than reported in the literature. The concentration addition model described quite well the mixture effect. Basic ecological risk assessment showed a potential risk of IMD and CLO for collembolans. These results are in line with many recent studies questioning the use of neonicotinoids in agriculture.

WE121

Toxicity of Neonicotinoids Towards Two Soil Invertebrates

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The present study aimed to answer three questions: (i) Does the toxicity of three different neonocotinoids (thiacloprid, imidacloprid and thiametoxam) differ between different species of soil invertebrate (*Folsomia candida* and *Eisenia andrei*)? (ii) Is there a difference in the toxicity between the neonicotinoids? (iii) Is there a difference in the toxicity of thiamethoxam and imidacloprid when dosed as pure compound or in the formulations Actara® and Confidor®, respectively? Ecotoxicological tests following OECD guidelines 232 and 222 were performed in order to assess the toxicity of the compounds to the springtail *F. candida* and the earthworm *E. andrei* after 28 days exposure in a natural standard Lufa 2.2 soil. Thiametoxam (pure compound and formulation Actara®) was the most toxic neonicotinoid for survival of *F. candida* (LC_{50s} 0.36 and 0.4 mg/kg dry soil, respectively), while imidacloprid and thiametoxam had a similar toxicity for reproduction of this springtail (EC_{50s} of 0.26-0.30 and 0.26 mg/kg dry soil, respectively). For *E. andrei* imidacloprid (pure compound and formulation Confidor®) was the most toxic neonicotinoid to for both endpoints (LC_{50s} of 0.80 and 0.74 mg/kg dry soil and EC_{50s} of 0.40 and 0.50 mg/kg dry soil, respectively), while thiametoxam, both pure and formulation, did not affect earthworm survival and reproduction at the concentrations tested (up to 10 mg/kg dry soil). Thiacloprid was the least toxic neonicotinoid for *F. candida* and *E. andrei* with LC_{50s} of 4.2-5.2 and 9.3 mg/kg dry soil and EC_{50s} 2.4-1.5 and 0.7 mg/kg dry soil, respectively. Compared to survival, reproduction was more impacted by thiacloprid and imidacloprid in *E. andrei* than in *F. candida*. The neonicotinoids presented a difference in toxicity towards the two species tested, with thiametoxam being most toxic to *F. candida*, and imidacloprid to *E. andrei*, despite both compounds belonging to the same chemical family of N-nitroguanidines. There was no difference in toxicity between the formulation and pure compound for imidacloprid and thiametoxam. This study showed that there are only small differences in the sensitivity to neonicotinoids of springtails and earthworms. This is remarkable as the first is closely related to insects, the target of neonicotinoids, while the latter is not.

WE122

Does the fate, persistence and toxicity of a semi volatile hydraulic lubricating oil vary across Canadian soil types?

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Canada contains over 1 million kilometers of pipelines. Specialized pipeline equipment in Canada utilize hydraulic lubricating oils that are released to the environment with no containment or liners to protect soil. This results in contamination of soils with C10 to C34 petroleum hydrocarbons fractions. Petroleum hydrocarbons in surface soils have the capacity to breakdown via microbial degradation. An effective management strategy includes nitrogen and phosphorus amendments to accelerate the degradation process. Canadian soils display a range of characteristics like clay and organic matter content that affect the rates of biodegradation and the toxicity of petroleum hydrocarbons to soil organisms that provide ecosystem services. Collembola (*Folsomia candida*) are a widespread soil invertebrate inhabiting a range of soils in Canada, contributing to ecosystem services through the breakdown of organic matter. The first objective of this study was to assess the fate and persistence of a semi volatile hydraulic lubricating oil in soils under unfertilized and fertilized conditions similar to a southern Canadian spring and summer. Secondly, we also investigated the toxicity of the contaminated soils to a standardized soil ecotoxicology invertebrate, *F. candida*. Fertilized and unfertilized soil samples from numerous Canadian soils were collected weekly over a four month microcosms experiment and C10 to C34 hydrocarbon concentrations determined to assess the fate and persistence of the lubricating oil. Single toxicity tests with *F. candida* were conducted on numerous uncontaminated and contaminated Canadian soils to determine the effects of the lubricating oil on survival and reproduction. Preliminary results showed varying first order rate constants and toxicity to survival and reproduction across soil types.

WE123

Recovery of Earthworm Populations in Field Test Plots through Migration?

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Earthworm field tests are used as highest tier in the risk assessment for in-soil organisms. Differences in earthworm populations (population size and composition) of test item treated and untreated field plots are assessed at different timings after application of the test item, thus covering short-term effects (1 month after application) as well as medium to long-term effects (6 months, 12 months after application)including . If full recovery is observed after a one year trial period, the risk is estimated to be low (De Jong et al., 2006; EFSA, 2015). The test (ISO guideline 11268-3, ISO 2014) is typically carried out on plots with a minimum size of 10x10 m that are separated from each other by untreated strips. It is known that earthworms are capable of active dispersal (e.g. EFSA 2015) and it could thus be postulated that migration could lead to an overestimation of the recovery potential of earthworm populations when studying small experimental plots – especially when the distance to potential “source areas” for migration (untreated areas) in relation to the potential dispersal rate is much smaller than in real world field situation with much larger distances to untreated areas (field margins etc.). A series of field experiments was started in 2016 in order - to estimate the contribution of migration from untreated areas to the recovery of highly damaged earthworm populations and - to evaluate whether migration could be reduced by either setting up mechanical barriers at the plot borders or by separating the plots from untreated areas by strips of intensively cultivated soil. Results of these field experiments will be presented and discussed. **References:** De Jong, FMW., Van Beelen, P., Smit, CE. & Montforts, MHMM., 2006: Guidance for summarising earthworm field studies – A guidance document of the Dutch platform for the assessment of higher tier studies. RIVM, The Netherlands, 47 pp. EFSA [European Food Safety Authority], 2015: Draft Scientific Opinion addressing the state of the science on risk assessment of plant protection products for in-soil organisms. EFSA Panel on Plant Protection Products and their Residues (PPR). EFSA-Q-2011-00978, Parma, Italy. ISO, 2014: ISO Guideline 11268-3: Soil quality – effects of pollutants on earthworms. Guidance on the determination of effects in field situations.

WE124

Which are the key soil parameters driving the toxicity of chemicals to soil organisms?

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According to current guidance, risks for soil organisms exposed to chemicals (e.g. plant protection products (PPP), pharmaceuticals) are assessed in a first step based on standardised tests performed in artificial soils (e.g. [1, 2, 3]). These substrates are a mix of sand, peat, kaolinite clay and calcium carbonate and are not comparable to natural soils. Extrapolation from the endpoints determined in the laboratory to the field situation is currently performed by applying assessment factors, which should cover uncertainties regarding the goal of protecting the community of soil organisms in the field. However, limited data is available on the degree to which soil parameters influence the toxicity of chemicals in field soils, since toxicity is modulated by chemical sorption and bioavailability. Possibly, soil parameters like pH, organic matter content or texture interact with each other, and in different ways, in modulating toxicity of different compounds to different organism groups. This unraveled complex points to a poor prediction and a possible bias in estimating the toxicity of test chemicals for soil organisms in natural soils. To address this concern, dedicated research is needed on the mechanisms of toxicity of chemicals with diverging physical-chemical properties and modes of actions in different natural soils. A screening project has been initiated in fall 2016, assessing in a first step the toxicity for earthworms and collembolans of three different PPP active substances in five different soils. The selected test soils are characterised by different amount and quality of organic matter, different pH values, water holding as well as cation exchange capacities. The active ingredients selected display different sorption and degradation characteristics. The present contribution gives the results of the test trials and analyses their specific input to current conceptual models predicting the toxicity of chemicals for soil organisms in field soils. Beside this, the dataset will be included in a shortcoming analysis, helping to prioritize future research on this complex topic. [1] SANCO/10329/2002 (2002): Draft Guidance Document on Terrestrial Ecotoxicology. 17 October 2002. [2] OECD 222 (2016): Guideline for testing of chemicals – Earthworm reproduction test [3] OECD 232 (2016): Guideline for testing of chemicals – Collembolan reproduction test in soil

WE125

Derivation of species sensitivity distribution for bisphenol A for the protection of soil ecosystem

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Bisphenol A is used for making plastics, copy paer, and receit paper. However, lack of soil ecotoxicity data are reported till date. In this study, species sensitivity distribution for bisphenol A for the protection of soil ecosystem was derived by conducting multispecies toxicity test in LUFA 2.2 soil. For soil acute assays, eight species from six different taxonomic groups (nitrification microbes, soil algae, monocotyledoneae, dicotyledoneae, earthworms, and springtails) were assessed. For soil chronic assays, six species from five different taxonomic groups (soil algae, monocotyledoneae, dicotyledoneae, nematodes, and springtails) were tested. The produced data were evaluated, tabulated, computed and then used for the derivation of species sensitivity distribution for bisphenol A. In addition, the hazardous concentration at 5% species (HC5) from our test and previous researches were compared. This study suggested the criteria value of bisphenol A for the proteciton of soil ecosystem based on the species sensitivity distribution approach. *This study was funded by the Korea Ministry of Environment (MOE) as 'the Environmental Health Action Program (RE201603033).*

WE126

Terrestrial toxicity of silver nanoparticles using soil alga

S. Nam, J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science

There were no available toxicity data of nanomaterials to soil algae. In this study, we performed culture medium extraction method for assessing soil algal bioassay. Test species was *Chlamydomonas reinhardtii*. Multiple endpoints exposed to silver nanoparticles-contaminated LUFA soil were biomass, photosynthetic activity, cell morphology, cell membrane permeability, esterase activity, and oxidative stress. We verified effects of biomass, photosynthetic activity, cell size, cell membrane permeability, and esterase activity of *C. reinhardtii*. To the best of our knowledge, this is the first study to evaluate soil toxicity of nanomaterials using soil alga *C. reinhardtii.* *This subject is supported by Korea Ministry of Environment as the GAIA project (2014000560001) and by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2016R1A2B3010445).*

WE127

Assessment of effects of PAHs on soils functions in contaminated soils

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APPLICERA is a 3-year project aiming at developing a methodlogy for site-specific environmental risk assessment (ERA) of contaminated soils, where relevant soil functions (SFs) are included. Current ERA tools suffer from a limited understanding on how contaminants affect soil ecosystems and functions. This frequently leads to expensive dig-and-dump, where all soil masses with levels above guideline values are removed, but with largely unknown benefits for the soil ecosystem and functions. Within APPLICERA, one aim is to investigate contaminated sites *in situ* and test a battery of relevant indicators to explore how biotic and abiotic indicators can be used to express SFs. The selected site is a railway yard in southern Sweden, close to the city Mjölby. It is a property of the Swedish Transport Administration, and various activities have been ongoing there for more than 150 years. In a previous survey in 2010, potentially contaminated areas were identified due to a number of activities, such as depot for cross-ties, loading dock and locomotive stable. Here we present the results from a soil sampling in June 2015 from 15 locations from this contaminated area. The samples have been analyzed for a battery of chemical, ecological and eco-toxicological indicators indicative of the SFs. The contamination levels of three groups of PAHs (PAH_{16}, alkyl-PAHs and oxy-PAHs) were determined in the soil (total levels) and in the pore-water (bioavailable fraction), which is a more relevant measurement for correlation to potential toxicological effects on the micro-macro fauna. The soil has been characterized, metals measured and the micro- (nematodes), meso- (springtails and mites), and macrofauna (worms) counted and families determined. For relevant microbiological indicators, the enzyme coding genes present in groups of microorganisms performing specific functions have been analysed. These functions are key elements in e.g. the N-cycle, and can therefore potentially serve as functional indicators. A methodology for site-specific ERA will be developed within the project, where SF rather than biodiversity is in focus, i.e. a more direct methodology. The most promising microbiological SF indicators will be used together with other ecological, chemical, and ecotoxicological indicators in a site-specific TRIAD. Finally, consequences of a shift towards site-specific SF-based ERA methodology will be evaluated versus traditional approaches.

WE128

Assessing the effects of biochar on the toxicity of the agrichemical imidacloprid on potworms (Enchytraeus albidus)

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Enchytraeids (Oligochaeta) are important soil promoters and provides various benefits to plants. Once in nature, imidacloprid threatens various non-target organisms. Biochar has been suspected to attenuate the efficacy of pesticides. The present study aims to find out whether biochar has the potential to alleviate the effects of pesticides on non-target soil organisms. *E. albidus* was exposed to a geometric series of imidacloprid concentrations in the OECD (Organisation for Economic Development) soil for 21 days. To assess the effects of biochar on the toxicity of imidacloprid, *E. albidus* was also exposed to biochar amended (10%) OECD soil. Because imidacloprid is an agro-pesticide, a germination experiment using wheat seeds was performed to evaluate the effect of imidacloprid on plant germination. For each wheat cultivar, the treated seeds had significantly longer coleoptile than the non-treated seeds ($P \leq 0.05$). Imidacloprid caused a significant decrease in the reproduction of *E. albidus* in both soils. However, a higher EC_{50} (46.23 mg/kg) in the biochar amended soil than in the non-amended one (EC_{50} = 22.27 mg/kg) indicated a decrease in the toxicity of imidacloprid in presence of biochar. For both soils, significant mortality was only observed at the two highest imidacloprid concentrations ($p \leq 0.05$). No significant difference in mortality between the two soils. These findings indicate that Imidacloprid could enhance plant germination but it could have negative effects on the reproduction of non-target soil organisms such as enchytraeids. Biochar could be used as the soil amendment to reduce the toxicity of imidacloprid and other such chemicals in soils.

WE129

SETAC Soils Interest Group

M.H. Wagelmans, Bioclear earth

WE130

The gut wall of the earthworm Eisenia fetida harbours a closely associated bacterial community distinct to the soil community

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The microbiome can play an essential role in the heath of its host. When the interaction between host and microbiota is disturbed by for example environmental pollutants, negative effects on the host can be expected. Earthworms are vital to the functioning of the soil and thus disturbance of these animals can have a major impact on the whole ecosystem. The microbial community associated with earthworms has been relatively well studied. A large proportion of this microbial community is however transient and therefore might not have a strong biological interaction with its host. This study aimed to assess the non-transient bacterial community closely associated with the gut wall of the earthworm *Eisenia fetida*. Using Illumina 16S sequencing the bacterial community was assessed in culture soil, cast, non-depurated guts, depurated guts and depurated guts that were washed with different intensities. Multivariate analysis showed that the community in the soil, cast and the non-depurated gut was distinct to the more non-transient community found in the depurated and washed guts. In soil dominant phyla such as *Verrucomicrobia*, *Acidobacteria* and *Chloroflexi* were hardly present in the depurated and washed guts whereas the phyla *Tenericutes* and *Spirochaetes*, present in low abundances in the soil, were dominant in depurated and washed guts. The species diversity was the highest in the soil and significantly lower in other sample groups, especially in the depurated and washed guts. Most observed taxonomical units found in the gut communities were also found in the soil, but there was a small but consistent population that was gut specific and not found in the outside environment. Highly abundant bacterial taxa consistently present at the gut wall included among others *Vermineprhobacter spp.* and *Spironema spp.* and unidentified species belonging to the families *Cytophagaceae*, *Aeromonadaceae* and *Microbacteriaceae*.The data show that the community profile of the depurated gut was in between the soil and the washed gut community indicating that depuration of earthworms alone is not sufficient to extract a gut wall specific signal. It is expected that the closely associated community described in this study might play a role in the health of its host. Future work will focus on how this community responds to environmental pollution and whether such community changes can have indirect health effects on the host.

Challenges and best practice in monitoring of micro- and nano-plastic abundance and environmental distribution (P)

WE131

Abundance and characteristics of microplastics in bivalves and lugworms from urban, aquafarm and rural areas of the Korean coasts

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There are increasing observations of microplastic ingestion by marine organisms representing various trophic levles including seabirds, marine mammals, fish and invertebrates. In particular, microplastics can be ingested by low trophic level species such as lugworms, amphipods and barnacles, mussels, and zooplankton due to their small size. Bivalves and lugworms have been widely used as biological indicators of environmental pollution in various monitoring programes by virtue of their broad geograhical distribution, easy assessiblity, high tolerence to salinity, and limited mobility. Similary, bivalves can be useful bioindicators for assessing microplastic pollution in water colume because they filter large volumes of seawater, while lugworms can reflect microplastic pollution in bottom sediment. In order to assess the contamination status of microplastics in marine invetebrates and their contamination characteristics according to diffent feeding habitats and regional sources, mussels (*M. edulis*), oysters (*C. gigas*), and lugworms (*Perinereis aibuhitensis*)were collected from aquafarm, urban and rural areas in the southern coast of South Korea. Microplastic pollution was widespread in three marine invetebtate species inhabiting the coastal waters of South Korea. Total 1346 microplastics were identifyed from 103 individuals using µFT-IR. Interspecies and regional differences was observed in the levels and polymeric compositions of ingested microplastics. Lugworm contained significantly higher amounts of microplastics than bivalves. PE and teflon was dominant in mussels and oysters, while teflon was dominat in lugworm. High concentraion of microplastics was found in organisms from aquafarm area. Diverse kinds of polymers were identifyed from urban and aquafarm areas compare to those from rural area. Most dominant types of microplastics were white (or colorless, 97%) in color, less than 300 µm in size (>80%), and fragment (96%) in shape. Identification of polymer type is essential process for microplastic analysis. Especially, fiber type of microplastic can be overestimated without spectroscopic analysis.

WE132

Spatiotemporal Distribution and Source of Microplastics on Korean Sand Beaches

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Microplastics are widespread and ubiquitous in the marine environment. Especially, beach is the most important location of microplastics accumulation. Contamination level of microplastics in various marine compartments were widely reported in the world, but nationwide assessment of microplastic pollution wes limited. Spatial distribution within a beach is not well addressed yet. Major factors influencing on pollution levels and spatiotemporal distribution of microplastics on beach are still unclear. Multiple lines across a beach (backshore: B, strandline: S, middle: M, and wateredge: W) and two seasons (before and after rainy seasons) were investigated to evaluate spatiotemporal distribution in twenty beaches along the coast of Korea in 2016. The relationship of microplastic abundance with environmental and potential source related factors influencing their spatial distribution were evaluated. The median value of microplastic abundances at each sampling line was in order of B > M > S > W. The mean abundance was significantly (Tukey HSD, $p < 0.05$) higher in B line than M and W lines. Among 20 beaches investigated, B and S lines showed the highest abundance at 11 and 9 sites, respectively. Sampling of microplastics from only high strandline may overestimate microplastic pollution level or miss the hot spots near backshore. Therefore, mutiple-line or transect survey is required to obtain representing sample from a beach. The mean abundance of L-MP in after rainy season was 10 times significantly (*t*-test, $p < 0.05$) higher than that in before rainy season. It is also required to select appropriate sampling periord regarding the seasonal variation. L-MP distribution deomonstrated significant (Pearson correlation, $p < 0.05$) positive correlation with S-MP, beach width, population, and proximity to the city among the factors tested, on the other hand none of significant correlations were obtained between S-MP and all the factors other than L-MP. In this study, significant increase of L-MP abundance after rainy season and correlation with the land-based sources imply their main input source is allochthonous. Main input source of S-MP may be considered as autochthonous because of significant correlation with L-MP and none of correlation with the land-based sources. Keyword: Microplastic, Spatial distribution, temporal distribution, source.

WE133

Microplastics and their Alternatives for Intentional Use in Products - An Application for FT-IR Micro-Spectroscopy

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Microplastics in cosmetic and personal care products are predominantly known for their use as an abrasive. However, their use extends far beyond this function: they can ensure a delicate, silky texture or improve the product stability. In more technical terms they are added for viscosity regulation, emulsification, film forming, binding or as a bulking agent. Polymer waxes are, for instance, found in paints and coatings. There is no international standardised definition of the size and composition of microplastic. It is mostly agreed that microplastic particles are of size below 5 mm and consist of synthetic petro-based polymers. Some open questions still under discussion are which synthetic polymer types should be excluded (e.g. bioplastics produced from renewable sources, rubbers), and how to rate (water) solubility and biodegradability. To make it more complex, a given polymer may appear as solid particle, wax or even liquid depending on the molecular weight and crystallinity. The often used term 'microbead' cannot be used interchangeably for microplastics, because it usually refers to the microplastics' function as an exfoliant or cleanser in rinse-off cosmetic and personal care products. In fact, there are plenty of leave-on products (e.g. decorative cosmetics, sun cream) where plastic particles are added for other than abrasive functions. There is evidence that all of the world's biggest consumer goods companies have used or are still using microplastics additives in their products. Most beauty brands, however, pledge to end the use of exfoliating plastic microbeads. The relevance of microplastics in household and industrial care products, in paints, varnishes and other specialised applications cannot be quantified yet. We will present an overview of intentionally added microplastics in products. This includes information on the polymers used in different applications, their functions as well as non-plastic alternatives. A more analytical perspective will be covered by application examples of FT-IR micro-spectroscopy and imaging. We will compare different measurement possibilities in order to confirm the microplastics listed as product ingredient, and will give examples for alternative substances found.

WE134

Characterising physical- and photodegradation of polystyrene and polyethylene microplastic particles

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We investigate the physical- and photodegradation of microplastic (MP) particles and attempt to understand which MP physicochemical properties exert the most significant influence on these processes. Polystyrene (PS) and polyethylene (PE) MPs are used to study the effect of polymer composition on degradation, whilst particles of ~10 μm and ~200 μm are used to investigate the role of particle size on degradation. The 10 μm MPs are synthesised at this size range as model spherical reference materials. The 200 μm MPs are produced by cryogenically milling pristine low density PE (LDPE) and general purpose PS (GPPS) pellets. The physicochemical properties of all four MPs were comprehensively characterised prior to use and methods developed for monitoring changes in these properties during the two different degradation studies. Scanning electron microscopy is used to provide information about MP particle size, shape and morphology. Pyrolysis GC-MS is used to identify the main chemical additives present in each of the test MPs. ATR-FTIR was used to verify the polymer composition of each material and provide a reference point for studying photochemical transformation. Physical degradation is studied by placing the MPs in conical flasks containing sterilised seawater and reference sediment. Samples are placed on an orbital shaker (150 rpm), in the dark for up to 56 days. Prior to analysis, the MP fraction is isolated using density separation. The reference sediment is pre-treated with the density separation method to minimise the presence of inorganic particulates in the MP fraction. Changes in the average particle size distribution are determined using optical light microscopy and manual counting (200 μm particles) or by Coulter Counter (10 μm particles). Photodegradation studies are conducted by placing the MPs in custom-made quartz tubes with sterilised seawater. Samples are fixed on a rocking table located directly beneath an Atlas SUNTEST CPS+ instrumented fitted with a xenon lamp and sunlight filter (UV and visible; 280-800 nm), and exposed continuously at 600 W/m^2 for up to 56 days. Following isolation by filtration, the MPs are analysed by ATR-FTIR in order to study changes in the surface chemistry resulting from photochemical transformation. In both degradation studies, pyrolysis GC-MS is used to investigate the degree of additive leaching through studying changes in the ratio between additive peaks and those of the main polymer matrix.

WE135

Determination of plastic loads in an urban river

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A reliable quantification of the occurrence of plastic material in the aquatic environment is an essential prerequisite for the assessment of potential ecological consequences and the development and implementation of mitigation measures. The occurrence of plastics in the marine environment has long been recognized and several studies provide quantitative data. Fewer studies have focused on freshwater systems. However, freshwater systems such as rivers may act as a transport pathway from the source of plastics into the oceans and/or as a sink and buffer of plastics. In the presented study, we sampled suspended and floating matter (with a focus on plastic material) in the Parthe River, (Leipzig, Germany) upstream (P1) and downstream (P2) of an urbanized area and investigated the change in plastic concentrations and loads. A floating drift net with a mesh size of 500 μm was used during each 24 h sampling campaign. Collected samples were fractionated according to the predefined size fractions of 0.5 to 1 mm, 1 to 5 mm, 5-10 mm, and > 10 mm. Plastic extraction from the smallest size fraction was achieved by chemical digestion and subsequent density separation. For the size fractions above 1 mm, plastic particles were separated visually. All extracted particles were subsequently analyzed by Raman-spectroscopy for identification of the polymer type. The load clearly increased with the degree of urbanization, as both, the number concentrations and the water discharge increased between P1 and P2. Mass concentration measurements indicated also higher concentrations at P2 compared to P1.Concentration-discharge relationships will be established. Furthermore, the input of plastic into the river will be related to the degree of urbanization at the study site. An appropriate assessment of plastic loads requires both, reporting of the particle and the mass concentrations. Bias is introduced by the sample preparation and analysis due to the selected particle size cut-off during sampling (> 500 µm). For quantification of plastic with particle sizes

WE136

Advanced microplastic clean-up for Raman micro-spectroscopy

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Monitoring microplastic particles in surface water requires sampling of large water volumes, typically several hundred litres to several cubic metres. Particle sampling goes hand in hand with particle concentration when a filter system is used. Particulate matter of environmental waters consist mainly of natural particles. The high number of natural particles compared to microplastic [1] can disturb the analysis. Besides problems with organic particles, microplastic can be covered by or burried under inorganic particles. To simplify the analytical detection of microplastic particles with Raman micro-spectroscopy, microplastic needs to be isolated from the particle concentrate. For particle sizes < 300 μm down to 1 μm or smaller, conventional density separation is not a suitable concept to remove the inorganic particles. Based on theoretical calculations and experiments with plastic and inorganic reference particles, an optimized method for the isolation of microplastic particles from inorganic particles was developped. The method uses sodium polytungstate solution and a centrifuge. After centrifugation, particles were concentrated on a filter and were analysed with Raman micro-spectroscopy for particle size and polymer type. Successful separation and particle recovery were achieved with reference particles. The method was applied to environmental samples which were taken and pre-concentrated with a self-made filtration apparatus [2]. Finally, particle concentrations in the environmental samples were calculated. [1] Storck, F.R., Kools, S.A.E., Rinck-Pfeiffer, S. (2015) Microplastics in Fresh Water Resources. GWRC Science Brief September/2015, Global Water Research Coalition, Stirling, Australia, 8 pp. Access: http://www.globalwaterresearchcoalition.net/_r1170/media/system/attrib/file/537/GWRC%20Science%20Brief%20Microplastics%20%28September%202015%29.pdf [2] Pittroff, M., Schmutz, B., Brauch, H.-J., Storck, F.R. (2016). Successful application of a cartridge filter cascade for sampling of very small microplastic particles in surface water and groundwater. Abstract, 7th Late Summer Workshop "Microplastics in the aquatic environment", German Water Chemistry Society, Haltern am See, September 25th - 28th, 2016, 4 pp. Acknowledgement: Dr. Oliver Köster, Dr. Oliver Happel, Beat Schmutz; German Fed. Min. of Education & Research (02WRS1378F).

WE137

Qualitative and quantitative analysis of microplastics in the marine environment - pyrolysis-GC/MS as a sensitive analytical tool

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There is a raising concern about plastic debris in the marine environment. The proportion of microplastics (MP) is expected to increase steadily due to ongoing fragmentation processes. Sources, distribution and accumulation behavior of S-MP (< 1mm) is comparably poorly understood due to restricted availability of data (Cózar et al. 2014). Identification and quantification of MP in the water column, sediment, and biota are comparably time-consuming and still missing standardization. Exclusively microscopically recognition and counting are losing reliability on a micron scale. Combined microscopic and spectroscopic FTIR- and RAMAN-techniques are the most established in MP analysis. Counting their size related abundances concurrently MP particles are identified via their polymer specific spectra. More recently imaging techniques became popular, which enable an almost automatic scanning of prepared samples for characteristic absorption bands of selected polymers. However, these techniques are time-consuming, because analysis time increases substantially with decreasing particle size. Pyrolysis gas chromatography mass spectrometry (Py-GCMS) as well as thermochemolysis are frequently used tools for the characterization of polymeric material. Until now both were mostly used for identification and rarely for quantification of single plastic types in natural samples. The advantages of this techniques are comparable short analysis times, a high sensitivity and the generation of quantitative chemical and weight related data (complementary to number and size related data). Our study applies Py-GCMS combined with thermochemolysis for simultaneous analysis of nine majority plastics (PE, PP, PET, PS, PVC, PC, PA6, PMMA, MDI-PUR). Selected fragments ions of specific pyrolysis products enable a sensitive polymer specific identification and quantification on a ng to μg trace level. Prior to Py-GCMS environmental samples need a multistep enzymatic, oxidative treatment and occasionally pre- or pursued density separation in order to achieve substantial MP-concentrations. The applicability of this method will be demonstrated on different environmental sample types. The quality and potential of this method will be checked with recovery experiments and potential matrix interferences will be discussed. Literature: Cózar et al. 2014. Plastic debris in the open ocean. PNAS 111(28), 10239-10244

WE138

Modelling the fate and transport of plastic debris in fresh waters - review and guidance

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Contamination with plastic debris has been recognized as one of today’s major environmental quality problems. Because most of the sources are land-based, concerns are increasingly focused on the freshwater and terrestrial environment. Transport and fate models for plastic debris can complement information from monitoring through measurements and will play an important role in the prospective risk assessment of plastic debris. We review the present knowledge with respect to fate and transport modelling of plastic debris in freshwater catchment areas, focussing especially on nano- and microplastics. Starting with a brief overview of theory and models for non-plastic particles, we discuss plastic-specific properties, processes and existing mass-balance-, multi-media- and spatiotemporally explicit fate models. We find that generally many theoretical and conceptual approaches from models developed earlier for other types of (low density) particles apply also to plastic debris. A unique feature of plastic debris, however, is its combination of high persistence, low density and extremely wide size distribution, ranging from the nanometer to the >cm scale. This causes the system behaviour of ‘plastic debris’ to show a far wider variety than any other material or chemical. We provide recommendations for further development of these models, and provide implications and guidance for how fate and transport models can be used in a framework for the tiered risk assessment for plastic debris.

WE139

To what extent are wastewater treatment systems a gateway for microplastic particles in the aquatic environment and in soil?

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Since the early 1950’s, there has been an increased production and use of plastic worldwide. The use of plastics has had major impacts on our everyday lives and led to decreased costs and increased safety in industries like construction and the food industry. However, it is also becoming more and more evident that this persistent material also represents a major emerging treat to the environment. Microplastics can be of either primary or secondary origin. Primary mircoplastic are purposefully manufactured to be of microscopic size such as plastic micro beads added to face wash, toothpaste, sunscreen and shaving cream formulations. Secondary microplastics are derived from the fragmentation of macroplastic items and waste from mechanical cleaning of synthetic polyester and other synthetic materials clothes. While knowledge about the fate and effects of microplatics in the marine environment are advancing, there are immense gaps of knowledge regarding freshwater. Data on their abundance is fragmentary or absent for small surface waters and terrestrial ecosystems. Relevant sources of microplastics and their environmental fate remains to be investigated. Large amounts of these microplastic particles are likely to be collected in the sewage system, and waste water disposal facilities have been identified as point sources of microplastic in the aquatic environment, but to date little is known about these facilities as possible contributors into the terrestrial ecosystem. Today's waste water treatment plants (STP) are not designed to manage microplastics. Preliminary studies have shown that a considerable, but varying, fraction of the microplastic particles arriving at a treatment plant are retained by the plant. More knowledge is therefore required concerning inlet/outlet flux estimation, chemical characterization, behaviour and fate of microplastic particles in different sewage treatment systems. The main objective of this research activity was to develop a standardized method to collect, quantify and characterize microplastic particles. This study will contribute to the knowledge about the possible significance of waste water treatment facilities as point sources of micro-plastic particles into food chains both in water and land. Special attention will be addressed to the sewage sludge, a valuable by-product of the sewage treatment process being adopted in agriculture production.

WE140

Ascidians as bio-indicators of micro-plastic and phthalates in marine environments

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Worldwide occurrence of micro-plastic in the marine environment is a major aspect of plastic pollution and an increasing threat to marine organisms and ecosystems. Ingested by different organisms, these particles can cause mechanical damage to tissue and release toxic chemicals into organism. One group of such chemicals is phthalate plasticizers, added to plastics during manufacture to soften and increase flexibility. Phthalates are found in many products and are potentially harmful, are known to bio-accumulate in organisms, and have been associated with endocrine disrupting effects. Therefore, scientific methods aimed at assessing phthalates accumulation in organisms will contribute to our understanding of its eco-toxicological impact. Our goal is to investigate the potential use of solitary ascidians (Chordata, Ascidiacea) as *in-situ* biological indicators of micro-plastic and phthalates. As sessile filter feeders, they filter high volumes of seawater and retain particulate matter. Ascidians have a wide global distribution in both polluted and pristine waters, and some are very successful invasive species. As so, they make ideal candidates for monitoring micro-plastic and their additives in a wide variety of marine environments. Our objectives are: (1) Develop analytical methods for detection of micro-plastic and phthalates in solitary ascidians; (2) Investigate the ability of solitary ascidians to accumulate micro-plastic and phthalates in polluted *vs.* non-polluted sites, and (3) Specify the organs in which micro-plastic and phthalates accumulate in the organism's body. By developing methods to detect and quantify micro-plastic and plastic additives, along with verifying the use of ascidians as suitable bio-indicators for micro-plastic and phthalates, this study will present a new and applicable tool for bio-monitoring these contaminates in the marine environment.

WE141

Microplastic contamination in gudgeons (Gobio gobio) from Flemish rivers

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Plastic pollution is continuously growing on a global scale and emerging as a major environmental hazard, it is currently evoking an increasing scientific attention towards the effects and impacts of this persistent danger. With the smaller sized microplastics (< 5 mm) shown to be omnipresent throughout the aquatic environment, the total extent of the problem remains unanswered. The ingestion of microplastics is adversely affecting many species, leading to intestinal blockage, hepatic stress and the transfer of adsorbed environmental pollutants. While the occurrence of microplastics has received a lot of attention in the marine environment, freshwater ecosystems and their biota have been largely unstudied. This study tries to expand the current knowledge on microplastics in freshwater systems by documenting the occurrence in the digestive system of fish from 15 different rivers at 17 different locations in Flanders, Belgium. Four rivers were found to have fish containing microplastics, however, no significant differences could be established between the sampling sites. In total 78 different gudgeons (*Gobio gobio*) have been investigated, 9% of which had ingested at least one microplastic item. Microscopic and spectroscopic analysis showed the items to be from various sources, witnessing many different physical characteristics and finding seven different polymer types for a total of eight microplastic items found. Although further detailed research is needed, this primary study shows that gudgeons from Flemish rivers are contaminated with microplastics.

WE142

Visual sightings of floating debris in the Mediterranean Sea

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Anthropogenic debris is a growing environmental concern, whose implications are not yet thoroughly understood despite its global importance. In recent years, the role of plastic as the primary component of anthropogenic debris has come to the attention of the scientific community, and the ubiquity and potential effects of plastic debris are now being disseminated to the wider public and government bodies (e.g. EU Commission, 2011). Specific aspects are addressed in various legislations, like the Waste Framework Directive with its 50% household waste collection target by 2020. The Marine Strategy Framework Directive (MSFD) sets the framework for Member States to achieve by 2020 Good Environmental Status (GES) for their marine waters, considering 11 descriptors; descriptor 10, focuses on marine litter, stating that GES is achieved only when "properties and quantities of marine litter do not cause harm to the coastal and marine environment" (2010/477/EU). Herein we report results from visual sightings of floating natural (NMD) and anthropogenic (AMD) macro-debris (> 2 cm) taken in the central part of the Mediterranean Sea. Data have been collected during a dual-use campaign onboard the Italian Navy tall ship "Amerigo Vespucci" which circumnavigated the Italian peninsula during May-June 2016. As suggested by EU JRC guidelines on monitoring marine litter in European Seas, the distribution, abundance and composition of floating marine debris were assessed using a 10 m fixed-width strip transect method. Over 88 h of transect counts were performed, for a total of 168 transect, covering an overall survey length of 1026.35 km. About 5500 debris items were counted during transects, 73.2 % of which were plastic fragments. Floating debris was found throughout the entire study area with densities ranging from 0 to 9205 items/km^2 and with mean abundances of 492 AMD items/km^2 and 77 NMD items/km^2 across all surveyed locations. Maximum AMD densities (> 3500 items/km^2) were recorded in the Southern Adriatic Sea while the lowest densities (< 50 items/km^2) were found in the Central Tyrrhenian and in the Sicilian Sea. Our study documents the ubiquitous presence of anthropogenic debris (for the most part plastic items) around the Italian peninsula and underlines the need to expand our knowledge about the main sources and fate of marine litter in the Mediterranean region. However, despite these concerns, no specific EU legislation addresses plastic waste in a strategic way.

WE143

Dispersion of microplastic in different media and quantification in peripyhton using flow cytometry and viSNE data analysis

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Microplastic (MP) is defined as plastic particles smaller than 1 mm in diameter and is a highly diverse emerging pollutant of global concern. It occurs ubiquitously in aquatic environments and in a wide range of chemical composition, density, and shape. Thus, MP can sink in natural waters, potentially accumulating in periphyton. Periphyton is a benthic microorganism assemblage consisting mainly of algae and bacteria that are embedded in a matrix of extracellular polysaccharide substances (EPS). Potential impacts on structure and function by MP on periphyton are poorly known. With the objective to analyse interactions of MP with periphyton, we first aimed to achieve controlled exposure conditions. Therefore we examined various media to disperse MP. Furthermore we developed a method to quantify MP within stream periphyton by flow cytometry. We used various polyethylene MP types differing in size (4, 10 and two MP types with 75 μm in diameter) and density (0.96 and 1.25 g mL^{-1}) and compared the time-dependent stability of the MP suspensions in water, Tween80, bovine serum albumin (BSA), glucose, fulvic acids and EPS extracted from stream periphyton. The measurements were carried out using a cell counter. For flow cytometry measurements, MP (4 μm, 0.96 gmL^{-1}) were dispersed in a suspension of stream periphyton and its abundance was determined using viSNE data analysis. While in water and fulvic acid, MP only dispersed after sonication and strong mixing, in Tween80, BSA and EPS MP were easily dispersed. As expected, MP with the density of 0.96 g mL^{-1} buoyed upwards in all media, while MP with the density of 1.25 g mL^{-1} sedimented. The size of the MP determined the temporal dynamics of the observed processes. MP with 4 μm remained dispersed more than 10 min and MP with 10 μm and 75 μm accumulated within 1 min and 30 seconds, respectively. Flow cytometry coupled with viSNE data analysis allowed not only detection but also quantification of MP in a highly complex sample. This is possible because fluorescence and scattering characteristics of MP and periphyton organisms strongly differ. Altogether, this work helps to guide experiments with MP under controlled conditions and adds knowledge to MP behaviour in freshwaters.

Environmental consequences of oil and gas extraction and transport (P)

WE144

Impact of hydrocarbon on coastal microbial communities

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To date studies of hydrocarbon biodegradation have focused mainly on simplified systems (e.g. pure strains, individual molecules), which are not representative of natural environments. As a result, little is known about whole microbial communities in their actual context, and the impact of environmental fluctuations on pollutant degradation and microbial community structure is insufficiently investigated. Notably, microbial communities in the environment are frequently exposed to varying levels of oxygen, and while the aerobic and anaerobic biodegradation of hydrocarbons is well documented, little is known as yet about degradation within the transitional zone from oxic to anoxic conditions. Oxic/anoxic oscillations are commonly found in nature as for example at water/sediment interfaces or in bioturbated sediments. This fluctuating environmental conditions influence the microbial dynamic and its role on organic compounds degradation such as hydrocarbons. It is thus important to determine the behavior of microbial communities in such oscillating conditions, especially the microbial populations involved on anaerobic metabolisms such as sulfate-reducing microorganisms. \nIn order to decipher the mechanisms underlying microbial community organization after an oil spill, we develop experimental ecology approaches to manipulate the microbial communities. Maintaining sediments under conditions as close as possible to those prevailing in the environment allowed to propose a scenario describing the petroleum influence on microbial communities. After an adaptive stage, the modification of the microbial community structure occurred, concomitant with the beginning of the degradation of hydrocarbon compounds, followed by a succession of bacterial community structures along the degradation process. Submitting sediments to different oxygenation regimes in bioreactors showed the influence of the oxygenation on microbial assemblages and hydrocarbon degradation, which was favoured by oxygenation after a period of anoxia. Surprisingly, sulfate-reducing microorganisms were not affected by oscillating conditions.\nApplying different strategies of experimental ecology, our studies highlight the functional redundancy of microbial communities involved in hydrocarbons degradation. Better understanding of the effect of pollutants and the behaviour of microbial communities in oscillating conditions is essential to build a global view of their fate in natural environments.\n\n

WE145

Exploring inter-species sensitivity to a model hydrocarbon, 2-Methylnaphtalene, using a process-based model

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The objective of this study was to compare inter-species sensitivity to a model narcotic, 2-Methylnaphthalene (2MN), on species distributed in different regions; and to generate new insights into ecological factors governing species sensitivities to narcotic compounds. We fitted a toxicokinetic–toxicodynamic (TKTD) model to survival patterns over time for 26 species using new data from this study and raw data from the literature. The goodness of fit, quantified by the root-mean-square-error, is typically extremely good ranging from 0 to 0.07 with an overall mean of 0.02. When studying patterns in inter-species sensitivity to 2MN, cumulative distribution fits (SSD) provided little insight into understanding the drivers accounting for the differences in sensitivities. This is because the range of no effect concentrations obtained for 26 different species showed little variation (mean: 0.0074 mM; SD: 0.007) regardless of the species taxonomic relatedness, feeding mechanism, or geographical distribution. We found that the incipient LC_{50} was only reached for 4 out of 26 species. Our results suggest that these assessment metrics do not explain the complexity of the species tolerances and that the elimination rate and the derived time to observe an effect on survival, might provide the means for depicting patterns in sensitivity and for better ecotoxicological testing. When comparing the time to observe an effect, differences among regions are distinguished. Mollusks and second trophic level species were the less sensitive ones. Coupling our results with fate and transport models offers the possibility for theoretical predictions on the toxicity of other narcotic compounds while reducing animal testing. We provide recommendations concerning experimental design. Improvement in exposure tests allows for better model parameters for measuring the sensitivity of the species and ultimately assess the risk of marine communities to oil contamination.

WE146

ERA Acute - A multi-compartment environmental oil spill risk assessment model

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Environmental Risk Assessments (ERAs) are a mandatory part of the oil and gas (O&G) industry's environmental risk management (ERM) of operations, fields and installations. ERAs also provide input to comparison analyses such as Net Environmental Benefit Analyses (NEBAs) of oil spills, improved understanding of risks involved and are useful in identifying knowledge and data gaps etc. ERA Acute is developed using updated methodology based on more recent research from oil spill events such as the Deep Water Horizon incident. Four compartments are included: Sea surface, shoreline, water column and seafloor. The methodology is grid-cell based and results can therefore be shown in a geographical information system (GIS) allowing the user to identify areas of high risk; for use in decision support and spill response planning and assess environmental risk acceptability. The first step is impact modelling. Calculations in the different compartments use the same framework of probability of exposure, probability of lethal effect given exposure, presence of vulnerable resources and probability of incidents occurring, whilst reflecting differences in mechanisms of action for acute oil spills. Calculations are carried out for numerous oil spill simulations covering several months if needed. ERA Acute uses three levels of detail for resource presence. The first level assumes the most sensitive resource is present in all cells, and its quantitative result is suitable for screening, data gap identification etc. The two next levels use resource data to identify specifically where impacts and risks to resources are highest. The most detailed level uses fractions of the VEC present and calculates expected impacts in the form of resource loss. This is suitable for more detailed studies, e.g. for sensitive areas, detailed decision making, regulatory purposes etc. Impacts are summarised in a series of steps that allow the investigation of results at several levels. The next step calculates lag- and restitution times, based on the impact calculated in step 1. The resulting resource impact factor (RIF) and other risk endpoints from the summarising steps combine the extent of impact and recovery time, which allows the use of ERA Acute endpoints in several ERM applications; such as ERA, impact assessment, NEBA and risk matrix decision support approaches.

WE147

Oil and Gas field decommissioning: Application of Net Environmental Benefit Analysis (NEBA) to Comparative Assessment to determine the best decommissioning approach

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Many Oil and Gas fields are now approaching the end of their life span and require decommissioning. It is vital that consideration is provided to understanding the potential consequences, both negative and positive associated with decommissioning of these fields. In the UK full removal of these fields is required under OSPAR Decision 98/3, however there is potential for derogation of this Decision for complex structures and in these circumstances a Comparative Assessment (CA) can be conducted of alternative disposal options. Consideration of safety, environmental, technical, societal and economic criteria is outlined in UK Oil and Gas UK Guidelines. Ramboll Environ have been appointed by multiple clients to undertake CA's of a range of O&G structures, including pipelines, manifolds, drill cuttings piles and platforms, inclusive of assessment of contamination. Sediment habitats surrounding the O&G structures are particularly at risk of contamination, with potential impacts on the ecosystem functioning and a range of Ecosystem Services (ES). A method called Net Environmental Benefit Analysis (NEBA) has been developed to understand both the relative benefits and harm associated with alternative decommissioning options and can be used to inform management decisions. For CA of the environment and societal criteria, Ecosystem Service (ES) assessment have been used. Integral to the ES assessment is the understanding of the service provided by habitats. A new method has been developed and applied based on a method by Bas et al (2016), which provides a holistic assessment of the ecological functioning of a habitat and can be used to understand the effects of chemical contaminants together with the impacts of other stressors associated with decommissioning, such as physical smothering. This presentation will outline these new methods, with illustration of how they have been applied to various case studies for considering alternative options for O&G decommissioning. References: Bas A, Jacob C, Hay J, Pioch S, and Thorin S. 2016. Improving marine biodiversity offsetting: A proposed methodology for better assessing losses and gains. J Environ Manage 175: 46-59 Oil and Gas UK. 2015. Guidelines for Comparative Assessment in Decommissioning Programmes.

WE148

Biological effects of crude oil and chemically dispersed crude oil in Baltic blue mussels (Mytilus trossulus)

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Concerns that a major oil spill occurs in the Baltic Sea causing widespread damage to the local ecosystem have grown during the last years due to markedly increased shipping of oil and refined products in this region. There is a lack of knowledge of the toxicity and applicability of the newest chemical dispersants in the low salinity low temperature environmental conditions of the Baltic Sea. In the present study the Baltic blue mussels were exposed to water accommodated fraction (WAF) and chemically (dispersant) enhanced WAF (CE-WAF) of crude oil i.e. the water soluble fraction of crude oil and crude oil treated with dispersant prepared in separate bottles by mixing 24h with artificial brackish sea water at 10°C. Blue mussels filter several liters of water per day thus being exposed to high amounts of pollutants causing increased stress response levels in mussels. Since pollutant concentrations in mussel tissues can exceed the ones in surrounding seawater, it was also hypothesized that bacteria inhabiting the gills and digestive gland harbor genes related to oil-degradation. Biomarker responses of molecular, cellular and tissue level were investigated to detect the sub-lethal effects of the WAF and CE-WAF after 24h and seven days of exposure. Gene expression of selected stress proteins, reproductive status and sex were studied at molecular level with quantitative PCR (qPCR). In addition, the response of bacteria inhabiting gills and digestive gland was studied by measuring oil-degradation gene levels by qPCR. Biochemical methods were applied to study the antioxidant enzyme activities, neurotoxicity and lysosomal membrane stability. Concentrations of oil compounds in water and their accumulation mussel tissue were detected by chemical analysis. Oil concentration in the CE-WAF was approximately five times higher compared to WAF (3300 and 680 μg/L, respectively). According to the preliminary results the measured biomarker responses showed different response patterns in the WAF and CE-WAF treated mussels. Changed gene transcript levels of stress proteins and decreased lysosomal membrane stability was detected in the CE-WAF treated mussels. Antioxidant response seemed to be more altered by the WAF exposure. After completing all the biological and chemical analysis the data will be used to calculate an integrated biological response to provide scientific background for assessing dispersant use as an optional oil spill combating method in the Baltic Sea.

WE149
Biomarker responses to chemically dispersed crude oil in the Baltic Sea sediment dwelling bivalve, Macoma balthica
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In the Baltic area, oil spills are mainly combatted using mechanical collection of oil slicks. This method may be insufficient in high waves, or difficult ice conditions. The remaining oil is degraded by microbial activity, which can be a slow process in the subarctic conditions. The residual oil can sink into the sediment, and its fate and effect on biota is poorly known. Chemical dispersants, consisting of mixtures of solvents and surfactants, increase the oil bioavailability to oil degrading bacteria. Dispersants are used as an alternative method of combatting oil spills. The use of dispersants in the Baltic area is restricted by a HELCOM recommendation, partly because the first generation of dispersants were highly toxic. Currently used dispersants are less harmful to biota, albeit their use is not unproblematic, since the dispersed oil mass in water may cause short-term acute toxicity in higher marine biota. Despite this, the International Maritime Organization has created new guidelines for dispersant use, due to the successful dispersion of oil slicks approaching sensitive shorelines at the 2010 Deepwater Horizon oil spill. The pressure to use dispersants exists also in the subarctic area, as oil transportation constitutes a large part of the marine traffic of the biologically sensitive Gulf of Finland. We studied the effect and fate of dispersed crude oil in an aquarium scale experiment. The conditions simulate Baltic sea conditions, with low salinity and temperature. An array of enzymatic, neurotoxic, and membrane peroxidation biomarkers were performed using the sediment-burrowing bivalve *Macoma balthica*. The fate of the dispersed oil is estimated by quantifying PAH (polycyclic aromatic hydrocarbon) compounds and C10-C40 petroleum hydrocarbons from bivalve tissue and sediment. Overall, the 1:40 water to oil ratio did not increase the biomarker levels a great deal, and very little difference between dispersed and non-dispersed treatments could be seen. It is likely, that the sediment buffers some of the adverse contaminant effects, even though *M balthica* filtrates water, and any of its concurrent contaminants, for food. Further studies will be carried out to elucidate the effect of adsorption and sediment-dwelling oil degrading microbial life in the surrounding sediment.

WE150
Toxicity assessment of a naphtenic North Sea crude oil using multi-level endpoints in zebrafish early live stages
S. Johann, Institute for Environmental Research Biology V; L. Nuesser, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis; T. Seiler, RWTH Aachen University / Ecosystem Analysis
Steadily increasing oil consumption necessitates the development of risk assessment strategies covering future concepts for oil spill response. The EU-funded project GRACE focuses on diffuse oil spills in cold climate and ice-infested areas of the Baltic Sea and the Northern Atlantic Ocean. Crude oil is one of the most complex sample types for ecotoxicological investigations as it consists of thousands of compounds with widely varying physical-chemical properties. Hence, several common methods and tools have to be modified to gain a representative and reproducible picture of the toxicity. In this study, a regional representative naphtenic North Sea crude oil was used to evaluate oil spill and response impacts on biota by means of in-vitro effect-based tools. The acute toxicity of water-accommodated fractions (WAFs) on zebrafish (*D. rerio*) embryos and larvae was investigated using optimized exposure scenarios. Furthermore, the swimming behavior of larvae at the age of 96 hours post fertilization was evaluated in a light dark transition test to assess avoidance and neurotoxic effects. Focusing on more mechanism-specific toxicity, the oxidative stress response and genotoxic effects via the induction of micronuclei were investigated using zebrafish larvae or a zebrafish liver cell line (ZFL). Further experiments in the project will examine ecological relevant target species at a regional scale (bivalves, crustacean) and additional sensitive biomarker endpoints. Based on the toxicity data of model and regional organisms, species-specific direct links between molecular events and effects on the organismic level (adverse outcome links (AOL)) will be established to contribute to the whole project's challenge of oil pollution assessment by identifying novel relevant and sensitive biomarkers.

WE151
Application of time variable toxicity model to single hydrocarbons and petroleum substances
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Many aquatic exposures to chemical contaminants are variable in time and space. However, effects data used as the basis of environmental risk assessment are often based on continuous exposures for defined periods of time. The General Unified Threshold Model of Survival (GUTS) modeling framework offers a convenient toxicokinetic/toxicodynamic framework for interpreting standard toxicity data, and time variable toxicity data and can help fill this knowledge gap. Application of this framework to existing datasets developed under standard conditions (e.g., constant exposures over 2-4 d) identified typical ranges for key model parameters: killing rate, elimination rate, threshold for effects. This framework was extended to toxicity data for exposures of complex petroleum substances (e.g., crude oil, fuel oils) to aquatic invertebrates and fish using the hydrocarbon block method (HBM) as implemented in the PETROTOX model. Modeling of the available datasets indicated that toxicity was successfully described by assuming additivity of the fractional contribution of each block to the observed toxicity over time. Available short- (2-4 d), and long-term (14+ d) exposures were analysed to help understand the relative importance of stochastic death, and individual tolerance on the mode of toxicity for these exposures.

WE152
Transcriptome analysis reveals disruption in gene networks related to membrane integrity and metabolic processes in Silurana (Xenopus) tropicalis embryos exposed to mixtures of naphthenic acids
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Naphthenic acids (NAs) are carboxylic acid mixtures naturally occurring in crude oil and are considered to be emergent contaminants. NAs have been found in concentrations of up to 130 mg kg^{-1} dw in sediments after oil spills. Degradation of the oil hydrocarbons after the Deepwater Horizon oil spill in the Gulf of Mexico led to their conversion to carboxylic acids. NAs are also found in oil sands process-affected water, which is produced in Canada's Oil Sands region in Alberta. The aim of this project is to assess the morphological effects and transcriptomic responses in developing *Silurana (Xenopus) tropicalis* embryos following exposure to two oil-derived commercial extracts (S1 and S2) at a sub-lethal concentration of 2 mg/L. Exposure to both NA mixtures resulted in embryonic abnormalities that included edema, and cardiac and gut abnormalities. Exposure to NAs also decreased morphometric parameters, such as total length, tail length, and interorbital distance. Microarray analysis using a custom 4 x 44 K Agilent platform showed that 3,376 common genes were differentially expressed in embryos exposed to S1 and S2 Vs control ($p < 0.05$). Gene set enrichment analysis revealed that 20 biological processes, 18 molecular functions, and 18 cellular components were significantly enriched after S1 and S2 exposure ($p < 0.05$). Some of the pathways enriched were related to the observed abnormalities and included edema, gastrointestinal system, and cartilage differentiation. Other notable pathways affected by NAs included metabolism, cell membrane depolarization, and xenobiotic clearance. These data imply that NAs have multiple modes of action during developmental toxicity in amphibians. Efforts are underway to characterize NA composition to better correlate chemistry to the biological effects observed.

WE153
EU H2020 project GRACE: Experimental design for assessing the impact of

oil and oil-dispersant exposure on Norwegian Sea and Baltic Sea mussels (Mytilus spp.) under "natural" conditions

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The EU Horizon 2020 project GRACE (Integrated oil spill response actions and environmental effects) targets at exploring environmental impacts and benefits of a suite of marine oil spill response tchnologies in cold climates and ice-infested areas in the Norwegian Sea (NS) and the Baltic Sea (BS). As a part of the project, the impacts of oil and oil-dispersants on biota using biomarkers are studied to gain sound science-based knowledge about the effects of oil contamination in these regions. Biological effects of water-accomodated fractions (WAF) of oil and oil-dispersant mixtures will be tested experimentally in the laboratory using mussels (*Mytilus* spp.). The purpose of these experiments is establishing adverse outcome links (AOLs) by combining conventional biomarkers, high throughput histopathology and ecotoxicogenomics and multivariate analysis. The experiments will be conducted within a range of different temperatures and salinity conditions matching those occurring in the study areas at different times of the year. Likewise, different *Mytilus* species will be used: *M. edulis* in NS and *M. trossulus* in BS, although specie will be confirmed through molecular analysis as ther might be population shifts and hybridization. WAF, both from oil (Troll B) and from oil-dispersant cocktails will be prepared at 10ºC using consensus procedures and conditions. However, exposure conditions will vary between 5 and 15ºC as in the field and salinity will be 33-35‰ for NS experiments whilst it will vary between 5 and 15‰ in BS experiments; thus different dispersants will be tested as they are differently employed depending on the salinity (Finasol 52 in NS and Finasol 51 in BS). the hydrocarbon profile and levels in seawater will be determined in WAF at different times during exposure in seawater, and bioaccumulation in mussels and detoxification will be investigated as well. Standard procedures for exposure experiments, sampling and sample processing are proposed.

WE154

Using GUTS to explain dynamic mortality patterns for a marine copepod exposed to dimethylnaphthalene

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The marine ecosystem is exposed to stressors such as pollution resulting from oil and gas exploration. Mechanistic models are needed to interpret the effects of such stressors, and to predict the impact on the life histories of selected species under realistic environmental conditions. Toxicokinetic-toxicodynamic (TKTD) models are well-suited for this task as they provide a mechanistic link between external concentrations and effects on life-history traits. For the endpoint survival, almost all existing TKTD models can be seen as special cases of the GUTS framework; the General Unified Threshold model for Survival. Here, we apply GUTS to the effects of a single oil component, 1,3-dimethylnaphthalene, on the copepod *Calanus finmarchicus*. Calanoid copepods form an important part of the marine zooplankton, and *C. finmarchicus* is a common species in the Northern Atlantic Ocean and expanding up into the Arctic. Rather than a single TKTD model, GUTS is a framework from which specific models can be derived. However, selecting the 'most appropriate' GUTS model can be challenging. Our data set includes effects on survival as well as body residues over time, and is therefore well suited to compare different model cases. In this contribution, we present the different death mechanisms included in GUTS, and demonstrate how they explain the patterns in the current data set. Further, we discuss the consequences of using a specific model case for extrapolations.

WE155

Alteration of nuclear excision repair pathway by crude oil exposure revealed by C. elegans transcription factor RNAi screening and zebrafish toxicity test

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Various (eco)toxicity and exposure studies have been conducted after Hebei spirit oil spill accident in Taean, Chungnam, South Korea, December 7, 2007. However, in-depth toxicological mechanism studies on spilled crude oil are still limited. Here we investigated mechanism of toxicity of the crude oils in two model organisms, i.e. nematode, *Caenorhabditis elegans* and zebrafish, *Danio rerio.* Our strategy is to start with *C. elegans* transcription factors RNAi library screening test to gain an insight of toxicity related pathways, and validate the selected pathways using *C.elegans* reproduction test with the selected gene RNAi. We further confirm the selected pathway in zebrafish embryo models. Iranian heavy crude (IHC) oil was used as it is the main oil components spilled during the accident..Age-synchronized RNAi fed worms were exposed to Water Soluble Fraction (WSF) of IHC oils for 72 hour and reproduction of RNAi fed worms was subsequently investigated by counting the number of off-springs from young adult using automated worm sorter, COPAS. RNAi-fed worms that showed increased or decreased sensitivity to IHC oils than control (empty vector-fed) worms were selected. The corresponding TFs, such as, T16H12.4 and T24H10.1, were identified as to be involved in protective or in toxic pathway to IHC oil exposure, respectively. Bioinformatics analysis was subsequently conducted on those TFs using KEGG and REACTOME database, which detected nucleotide excision repair (NER)with the highest significance (lowest p-value). To confirm this, we investigated the expression of NER pathway genes, such as, xpc-1, xpa-1, t16h12.4, ercc-1 using qRT-PCR in *C. elegans,* which was further confirmed in zebrafish. Increased expression of NER pathway genes was observed in both models, suggesting involvement of NER in toxicity of crude oil exposure. We further conducted the validation study with alkylated naphthalene, major components of IHC oils on both *C. elegans* and zebrafish models. Overall study suggests that 2007 Hebei spirit oil spill accident may cause bulk DNA damage in affected population, and as DNA damage may, in turn, lead serious health implications, such as, cancer, long term monitoring should be conducted on affected population. This study also suggests that *C. elegans* TF RNAi screening could provide a clue for predicting toxicity pathways of mixture chemicals, such as, IHC oils. *Keyword : Caenohabditis elegans,* RNAi screening, Zebrafish, Crude oil, Nucleotide excision repair, Mixture toxicity

WE156

Adverse effects of WAFs of crude oil on the Antarctic copepod Tigriopus kingsejongensis and the temperate congeneric copepod Tigriopus japonicus and identification of 30 cytochrome P450 genes

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Oil pollution has deleterious effects on marine ecosystems. However, the toxicity of crude oil towards Antarctic marine organisms has not been well studied. We compared the deleterious effects of water accommodated fractions (WAFs) of crude oil on reproduction, intracellular reactive oxygen species (ROS) levels, and antioxidant enzymatic activity in Antarctic (*Tigriopus kingsejongensis*) and temperate (*Tigriopus japonicus*) copepods. Reproductive rates of *T. kingsejongensis* and *T. japonicus* were significantly reduced ($P < 0.05$) in response to WAFs. Furthermore, *T. kingsejongensis* showed elevated levels of ROS and higher antioxidant enzyme (glutathione peroxidase [GPx]) activity than *T. japonicus* in response to WAFs. *CYP* genes from congeneric copepods were identified and annotated to better understand molecular detoxification mechanisms. We observed significant up-regulation ($P < 0.05$) of *Tk-CYP3024A3* and *Tj-CYP3024A2* in response to WAFs, suggesting that *CYP* genes may contribute to the detoxification mechanism in response to WAF exposure. These finding also suggest that WAFs induce oxidative stress, leading to reproductive impairment in copepods. Furthermore, *Tk-CYP3024A3* and *Tj-CYP3024A2* genes can be considered as potential biomarkers of WAF toxicity in the congeneric copepods *T. kingsejongensis*and *T. japonicus*. This study will be helpful for enhancing our knowledge on the harmful effects of WAFs in Antarctic and temperate copepods and provides insight into the underlying molecular mechanisms.

WE157

Direct Impact of Oil Pollution on Benthic Communities

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Human activities have been modifying the environment for decades, but many anthropogenic effects are poorly studied or even unknown. Petroleum and its derivatives are still a major source of fuel to human societies and, while acute impacts, like the *Deepwater Horizon* drilling rig explosion in 2010, still gets most of the public and environmental agencies attention, sources of chronic oil contamination represent the biggest anthropogenic input of petroleum hydrocarbons in the environment. Moreover, a variety of chemicals are added in the composition of lubricant oils, which are highly soluble compounds, thus posing a high risk for aquatic organisms. Freshwater and marine/estuarine benthic meiofauna (i.e. nematodes) communities were used to investigate the direct impacts of oil pollution on the aquatic environment. Nematodes are ubiquitous and among the most diverse metazoans both in marine and freshwater environments. They occupy key positions in benthic food webs, presenting different feeding types and life strategies, as well as different levels of tolerance to changes in environmental conditions, making them ideal organisms to investigate changes provoked by contaminants. Sediment was collected in the field and accommodated in aerated boxes for two weeks prior to incubation in experimental vessels. Freshwater and marine microcosm experiments were performed simultaneously, with a total incubation time of fifteen weeks. Nematode abundance in the marine microcosms started to decrease after three weeks, with no apparent recovery on the highest concentration treatments by the end of the assay. On freshwater microcosms, abundances decreased to a half after three weeks of initial exposure on the highest concentration treatments, compared to controls. Unlike the marine microcosms, there was an apparent recover on nematodes abundance after fifteen weeks. Species identification is ongoing but preliminary data (more than 60 species identified)

suggests a considerable decrease on species diversity on oil treatments compared to control replicates. The present study is 1) one of the first studies to assess the effects of oil-soluble compounds on nematodes and 2) one of the first assessments where marine/estuarine and freshwater/soil nematodes where compared.

WE158

Screening methods for assessing toxicity and fate of produced waters

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Due to their location there are significant logistical challenges with undertaking environmental risk assessments posed by offshore discharges. It is therefore important to be able to prioritise discharges where further investigations are warranted. As part of this process a 'toolbox' of simple screening tools have been developed and applied for the assessment of offshore produced water discharges. These require small, easily transported sample volumes providing quick and cost effective reliable information on the inherent hazard properties of the effluent (toxicity, hydrocarbon content, bioaccumulation potential) without extensive laboratory testing. These tools are typically applied to the first tier of a discharge assessment and include Microtox^{TM} testing, solid phase micro extraction with gas chromatographic analysis (SPME-GC) and Quantitative Structure Activity Modelling (QSAR) analysis. The obtained information, together with a dilution assessment, will be used to screen at an early stage and thereby decide whether any subsequent tier assessment is required. To date, 22 produced water samples from the North Sea, South-East Asia and Africa have shown that toxicity increases in line with bioavailable hydrocarbons, suggesting hydrocarbon contamination is a major contributor to effluent toxicity amongst other factors, and bioaccumulation potential is of low concern (BCF < 2000) even before taking into account biodegradation and volatilisation of BTEX components. Where higher tier assessments are necessary, a more detailed analytical characterisation of the produced water or a whole effluent approach, or both processes combined, will be required. The place of these tools within the tiered risk assessment framework is presented.

WE159

Comparative Risk Assessment (CRA) of Response Options for a Deepwater Oil Well Accident

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During the Deepwater Horizon accident, subsea dispersant injection (SSDI) was used as a countermeasure for this uncontrolled release from a deep water oil well. Since 2010, several studies have indicated that effectiveness of SSDI can be greater than dispersant treatment at the water surface. This paper summarizes a year-long comparative risk assessment (CRA) project, funded by industry and involving frequent feedback from the US regulatory community. The work involved 3-dimensional oil spill transport and fate modeling combined with data on ecological effects to evaluate comparative exposures to components of the ecosystem assuming various response combinations of SSDI application at the source, mechanical recovery of surface slicks, in-situ burning of surface slicks, and surface dispersant application. The objective was to provide decision makers with objective, science-based and transparent information to enable technically-sound choices regarding appropriate strategies for mitigating impacts from oil and gas released during a deepwater blowout. Given this context, an engagement process was established with stakeholder representatives, decision makers and others, throughout the project. The CRA evaluated potential tradeoffs from increased offshore and nearshore water column exposures of biota to dispersed oil and dissolved components in the water column resulting from dispersant use compared to increased exposures to oil on the water surface and on the shoreline without it. Oil spill trajectory model simulations were used to quantify the amount of shoreline, the area of the water surface, and the volume of the water column that exceeded defined exposure thresholds. Results demonstrate the importance of including SSDI among oil spill response technologies. SSDI can reduce the volume of oil that reaches the surface; reduce the volume of oil reaching nearshore ecological receptors; reduce exposure to volatile organic compounds (VOCs) for emergency response workers and biota; and reduce exposure of slow-to-recover wildlife to surfaced oil and VOCs.

WE160

Spill Impact Mitigation Assessment (SIMA) for Oil Spill Response Planning in the Arctic Environment

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Understanding the ecological consequences and mitigating effects of different oil spill response technologies is critical to the future of oil and gas exploration and production activities in the Arctic environment. Modeling the efficacy and effects of alternative spill response countermeasures is a powerful approach to inform decision-makers, because it quantifies the likely water volume adversely affected by naturally- or chemically-dispersed oil and dissolved hydrocarbons, as well as the surface area impacted by floating oil. This paper summarizes the results of a multi-year research project, funded by the oil & gas industry, to evaluate the ecological consequences of different spill response strategies and identify the set of strategies that could provide the greatest overall benefit for mitigating the environmental, socioeconomic and human health and safety impacts of release of oil in the Arctic environment. The assessment involved synthesis of nearly 2,500 research reports and scientific papers describing the ecological effects of oil exposure to Arctic marine wildlife. A wide range of information pertinent to assessment of mechanical recovery, in-situ burning, and surface dispersant application was compiled, including data on the fate and behavior of oil and treated oil in open water and ice conditions, the habitats and organisms that are potentially exposed, and the potential for effects and recovery following exposure. The approach provides decision makers with objective, science-based and transparent information for making technically-sound choices regarding strategies for mitigating the consequences of an accident in the Arctic environment.

WE161

Chemical and biological assessment of tight sand gas fracturing related waters

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Publicly available chemical risk assessments of hydraulic fracturing are generally based on shale gas related practices in the U.S., lacking other types of gas development also using hydraulic fracturing. This research focuses on polar organic chemicals present in hydraulic fracturing related waters from a tight sand gas development in the Netherlands. Fracturing fluid, flowback water samples and surrounding aquifers before and after the actual fracturing were analysed by means of HR LC-MS/MS, the Ames test and several CALUX bioassays. A suspect list (candidate chemicals) containing 881 chemicals was based on US and EU used and mobilised chemicals related to hydraulic fracturing. Less than half of these global candidate chemicals used in fracturing fluids are currently registered under European legislation. Considering that hydraulic fracturing in Europe only can make use of authorized chemicals, the number of possible chemicals is restricted compared to the US. In the fracturing fluid samples, 1009 different peaks are detected, including 10 that matched with the suspect list. 714 of these occur in concentrations – semi-quantitatively expressed as internal standard equivalent - exceeding groundwater thresholds based on the Threshold of Toxicological Concern (TTC) of 0.1 μg/L. 348 of these peaks are also detected in the flowback samples although at lower concentrations. In the flowback samples a total of 980 peaks were detected, including 19 that could be matched with the suspect list. Fingerprinting showed that 631 of these peaks originate from the subsurface. Between the first and eighth day of flowback, the number of peaks exceeding the TTC value drops from 291 to 189. In the groundwater samples there is no significant change in composition between the samples taken before and after the actual fracturing. In these samples, 50 peaks were detected with 12 exceeding TTC values. The Ames fluctuation test showed genotoxicity for some flowback and fracturing fluid samples. Furthermore, a selection of CALUX bioassay gave positive responses related to oxidative stress and P53 activity for fracturing fluids and flowback. The results point to the importance of the currently in place extensive measures related to the handling, transport and treatment of hydraulic fracturing related waters to avoid adverse environmental and human health impacts.

WE162

Oil spill and response impacts on biota in cold climates - effect-based tools and ecological risk assessment

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The EU H2020-funded project GRACE addresses oil spills in cold climate and ice-infested areas of the Baltic Sea and the northern Atlantic Ocean. Within a consortium of comprehensive expertise, the overall objectives are the improvement of marine oil spill detection, monitoring and oil spill response technologies. The work package introduced here (WP3) focuses on the particular environmental impacts of oil spills and response actions (in collaboration with WP4) on biota in these extreme environmental conditions. This will be achieved by examining ecologically relevant target species at a regional scale (bivalves, crustacean, fish) and the zebrafish as a well-characterized model organism in ecotoxicology. Regional specimen will be sampled from representative locations in the Baltic Sea and northern Atlantic. Bivalves will furthermore be sampled with respect to seasonal and longitudinal variation to establish biomarker baselines. Selected model oil types and commonly used dispersants as representatives for the study region and samples from oil biodegradation and remediation experiments (provided by WP2) will be investigated using bioassay batteries with sensitive biomarker endpoints. Based on the toxicity data species-specific direct links between molecular events and effects on an organism level (adverse outcome links; AOL) for the test organisms will be established. This approach is a useful tool to develop risk assessment strategies covering future concepts for oil spill response. Using the PETROTOX model and a data gap analysis, threshold values will be derived and trophic levels as well as test species revealed that are currently missing from the available regulations. This will feed in the experimental planning of the other tasks in the WP. Together with WP1, zebrafish-based on-line oil detection biosensors will be developed and analysed, which serve for background information to biodetection. The whole GRACE project contributes to the challenge of the prediction, the measurement and the assessment of the evolution of oil pollution.

WE163
Migration of polycyclic aromatic hydrocarbons in vertical profile of alluvial sediments of the Sava River, Serbia
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The plants for district heating in Belgrade (Capital of Serbia) have been using petroleum products as fuel for decades. The heating plant in New Belgrade is one of the largest heating plants in Belgrade Balcan. Due to its location in New Belgrade-alluvial plains of the Sava River, this heating plant represents potential source of the oil pollution for the whole alluvial area. Our previous research confirmed presence of different oil pollutants in these sediments (Miletic et al, 2015). The aim of our present research was to investigate the extent of vertical migration of polycyclic aromatic hydrocarbons hydrocarbons in vertical profile of alluvial sediments of the Sava River at this locality. In spring 2015, during an extensive analysis of the ground waters from the existing system of 10 piezometers, within the area of the heating plant in New Belgrade, three new wells were drilled down to the depth of 15 m. From these three new boreholes the soil and sediment samples were taken from several depths: 0-0.30 m; 0.50 m; 1.00 m; 1.50; 2.00 m; 5.00 m; 7.00 m, 10.00 m 12.5 m and 15.00 m. Pedologic analysis revealed that lithologic profile was represented by alternating layers of sand and clay. Moreover, most of the layers in the soil profile were characterized by low content of organic matter which results in a reduced adsorption capacity and reduced retention of oil pollutants (Delle Site, 2001). The soil and sediment samples were extracted for petroleum hydrocarbons with dichloromethane in a Soxhlet apparatus. The extracts were fractionated using column chromatography into fractions of: saturated hydrocarbons (Fraction I), aromatic hydrocarbons (Fraction II), and polar compounds (alcohols and keto compounds - Fraction III; Jovancicevic et al, 2005.). The aromatic fractions were analyzed by GC-MS techniques and comprised polycyclic aromatic compounds (PAHs) typical for crude oils and their derivatives. The results showed that low amounts of petroleum PAHs investigated were present in the whole profile investigated, down to the depth of 15 m. It can be concluded that in these alluvial sediments PAHs can migrate down to the depth of 15 m. Considering characteristics of the surrounding soil profile with low adsorption capacity and reduced retention of oil pollutants, it is necessary to raise awareness that these environmental pollutants can easily be remobilized and migrate to the surrounding soil, sediments and, probably ground water.

WE164
Vertical migration of oil pollutant in profile of alluvial sediments of the Sava River, Serbia
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One of the largest heating plants in the Balkans is located in Belgrade, the Capital of Serbia. Being located in the New Belgrade-alluvial plains of the Sava River, it represents potential source of oil pollution for the whole alluvial area. Presence of different oil pollutants in the soils and sediments in this area has already been confirmed (Miletic et al, 2015). The aim of our present research was to investigate compositional changes of oil pollutant during migration in vertical profile of the soil in this area. In spring 2015 an extensive investigation of this soil was conducted. The soil was sampled from 20 micro locations and at 5 different depths (down to the depth of 2 m) making in total 100 of samples. A manual Eijkelkamp auger soil sampling device was used, with the appropriate augers. The sampled material was arranged in layers and for each micro location a lithologic profile was made. Most of the layers in the soil profile were characterized by high content of sand and low content of organic matter. From these soil samples extractable petroleum hydrocarbons were isolated with dichloromethane in a Soxhlet apparatus. The extracts were fractionated using column chromatography into fractions of: saturated hydrocarbons (Fraction I), aromatic hydrocarbons (Fraction II), and polar compounds (alcohols and keto compounds - Fraction III; Jovancicevic et al, 2005.) In order to investigate compositional changes of oil pollutant during vertical migration, but also to examine the relationship between these changes and the composition of the surrounding soil environment, for each borehole investigated the group composition determined was plotted against the corresponding lithologic profile in each borehole. The results showed that in most of the extracts isolated polar compounds (Fraction III) were most abundant while saturated hydrocarbons (Fraction I) were least represented. This ratio between the fractions remained almost unaltered in different soil profiles in this area and at different depts. It can be concluded that in these alluvial sediments oil pollutants can migrate almost unaltered to the depth of 2 m. Additionally, it can be presumed that penetration of unaltered oil pollutants in deeper layers of soil at this location is the result of characteristics of the surrounding soil environment.

Environmental risk assessment in time and space - To boldly go where no man has gone before (P)

WE165
LIVING LAB: A NOVEL RESEARCH FACILITY TO CONNECT ECOTOXICOLOGICAL AND ECOLOGICAL RESEARCH
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Obtaining realistic predictions and measurements of how existing and emerging chemical stressors affect our natural environment is essential to provide the necessary tools to assess and mitigate ecosystem threats. While the basic idea is straightforward, attaining reliable estimates on the effects of chemicals on ecosystems has proved to be notoriously challenging. Historically, studies have focused on single ecosystems compartments. Recently, there has been an increasing recognition that anthropogenic pressures can resonate beyond the boundaries of single ecosystems, as exposure to emitted chemicals used at target sites may propagate to connected ecosystems. In addition, chemical impacts on an ecosystem level become more complicated through interspecific interactions throughout complex interconnected food webs. For instance, terrestrial consumers and predators can accumulate toxicants from adjacent water bodies with inherent implications for both bottom up and top-down effects within green and brown food webs. As a result, relatively little information is available on how chemical contaminants affects linkages between terrestrial and aquatic compartments, and hence novel experimental approaches are required. To facilitate answering pressing questions within this emerging field of research, a new research facility, called the 'Living Lab' has been constructed. This facility is composed of 36 ditches that are highly connected to a natural environment, thereby allowing natural processes (e.g. dispersal) to occur and to perform controlled experiments under naturally relevant, fluctuating conditions. We welcome research initiatives and collaboration within this novel research setting.

WE166
Traits-based analysis of macroinvertebrate community responses to insecticides using mesocosm data
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The high-tier effect assessment of pesticides is generally based on model ecosystem experiments in which worst-case exposure patterns are evaluated using species assemblages representative of freshwater ecosystems. The results of these experiments are generally interpreted using taxonomy-based approaches to derive threshold concentrations protective of effects on population abundances and community structure. An evaluation of these datasets based on physiological (e.g. respiration type) and ecological traits (e.g. voltinism, feeding type) would allow some advantages such as (1) the comparison and extrapolation of community effects across different geographic regions, (2) the elucidation of toxicity mechanisms leading to adverse effects, and (3) the linking between ecological effects and the functional diversity of ecosystems, elucidating impacts on the services/functions they provide. In this study we developed a methodological

approach to quantify the pesticide selective effects on community traits and tested it using a macroinvertebrate dataset obtained from a mesocosm experiment performed with the insecticide chlorpyrifos (Van den Brink et al. 1996). The proposed approach is based on the calculation of traits-based Principal Response Curves (traits-PRCs) and on the calculation of trait structure and functional diversity differences between samples using univariate techniques. The performed traits-based analysis yields less sensitive responses than the taxonomy-based analysis due to functional redundancy of species within a community. The calculations, however, show that the insecticide significantly reduces the prevalence of species with particular traits such as short life cycle duration, respiration by gills, small size and oviparity, which is in agreement with some previous studies performed using single-species toxicity data. So far the method has been developed and tested with one dataset, however we aim to test the method with other datasets derived from model ecosystem experiments performed with different exposure regimes and with substances with different mode of action in order to identify sensitive community traits associated to peak and continuous exposure patterns, and to different toxicological modes of action. The results of these analyses, besides the advantages described above, will help in the further classification of aquatic invertebrate communities regarding their sensitivity and vulnerability potential to chemical pollution.

WE167

Individual-based analysis of mayfly meta-population dyna

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Mechanistic models are gaining resonance as a means to bring more ecological realism and thus more informed effect predictions into the environmental risk assessment of chemicals, e.g. through interpolation and/or extrapolations of experimentally untested conditions. In particular, individual-based models allow the extrapolation of population level dynamics from individual organism behaviours and life history responses to environmental factors such as chemical exposure, thereby bridging the gap between traditional ecotoxicity observations and more relevant ecosystem level protection goals. We present an individual-based model where the life cycle of *Cloeon dipterum* individuals are described based on formulations provided by dynamic energy budget (DEB) theory. DEB models describe key life history processes such as growth, maturation and reproduction as functions of the energy gained from food assimilation. Moreover, in this model, we consider movement behaviours such as female search flights and oviposition habitat selection which influence spatial distributions of individuals. Just as in 'real life', modelled meta-population dynamics emerge from individual life histories, behaviours and interactions of individuals with their spatial explicit environment. We apply the model to investigate to what extent environmental factors trigger seasonal emergence patterns of mayflies and how selected landscape characteristics, such as the location of swarming markers and neighbouring freshwater habitats, influence meta-population dynamics. In a future step, the introduction of effects caused by stressors, e.g. chemicals, into the model could extend its use for environmental risk assessment.

WE168

A general modelling framework for environmental risk assessment - sublethal effects and starvation

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In current environmental risk assessment (ERA), plant protection products (PPPs) are tested on a diversity of standard test species for harmful effects. Recent developments in mechanistic effect modelling provide the possibility to extrapolate results from standard studies to untested species and untested ecological scenarios or exposure situations, which will improve the quality of ERA as well as saving time and resources. For lethal effects, toxicokinetic-toxicodynamic (TKTD) models have already proven the ability to identify patterns in effects across compounds and species. One crucial milestone of this development was the identification of a common modelling framework, the General Unified Threshold model for Survival (GUTS). This framework allows for using the same model for all species using different assumptions for death, which had previously believed to be conflicting. The Dynamic Energy Budget (DEB) theory may have the potential to provide such framework for sublethal effects. Models based on DEB theory allow for mechanistic interpretation of effects on feeding, energy expenditure, growth and reproduction. The same modelling framework can be used for all organisms, which is crucial for across -species extrapolation of effects. Models based on DEB theory have been used in ecotoxicology for decades, however, one major problem so far has been the ability of the models to predict the effects of starvation. Since many compounds act on the ability of the organisms to feed or assimilate energy, predicting starvation responses is a crucial characteristic for a TKTD model for sublethal effects to be used in ERA. We present here the first results of a project aiming to calibrate the DEB standard animal model in control conditions for a suite of vertebrate species, and to develop the starvation module for these species. In total, we are working on the standard DEB model plus starvation module for 11 species: two bird species (mallard duck, bobwhitequail), five mammal species (rat, mouse, rabbit, vole and woodmouse) and four fish species (rainbow trout, fathead minnow, zebrafish and medaka). We present a meta – analysis of the standard models of the fish, birds and mammals. We further discuss the potential impact using this general modelling framework in ERA.

WE169

Testing the BEEHAVE landscape module - complexity comes at a price

E. Zimmer, IBACON GmbH; T. Preuss, Bayer Ag / Environmental Safety

The honeybee colony model BEEHAVE (Becher et al., 2104) has been discussed as a potentially promising tool for modelling bee populations in ecologically relevant scenarios. However, according to the evaluation by the European Food Safety Agency (EFSA), some properties of the BEEHAVE model need to be more complex in order to fulfill the requirements to be used in ecological risk assessment. One of those properties is the representation of the landscape, which should include 'detailed spatial and temporal field data from defined study sites in Europe, and the contribution of these data to pollen, nectar and water availability, pesticide contamination and foraging behaviour.' (EFSA, 2016) In this poster presentation, we compare two conceptually different approaches for including a landscape in BEEHAVE. In a very simplistic approach, we use one flower patch with general properties that are constant over time to represent the whole landscape, which is similar to the approach that has been criticized by EFSA. We compare this approach with a realistic representation of the landscape, where we use Cropland Data Layers with a spatial resolution of 25 x 25 km. We combine the realistic landscape with spatially resolved weather data, and data on seasonal nectar and pollen availability, which represents the higher complexity required by EFSA. We validate both approaches with data from field studies conducted both in Europe and in North America, to additionally test the regional transferability of the model.

WE170

Spray-drift Exposure at Landscape-level - A Spatiotemporally Explicit Module and its Use in Ecological Risk Characterisation

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Spray application of plant protection products (PPPs) is generally accompanied by a fraction of the spray volume leaving the targeted field and depositing into neighbouring habitats of Non-Target-Organisms. The mechanistic process of spray droplets movement - from leaving the nozzle, their trajectory through the air and their deposition onto habitat elements - is complex. Therefore, with the aim to establish a robust basis for ecological risk assessment, estimation of spray-drift exposure is currently predominantly approached empirically. Rautmann has provided a collection and evaluation of spray-drift experiments which established an empirical exposure modelling. In this approach, spray-drift depositions onto habitats of Non-Target-Organisms occurring at the edge-of-the-field are represented by the 90th percentile depositions observed within and between field trials (at a given field-to-habitat distance). The variability occurring along the field edge (at a given distance) or between different applications is ignored. However, this local spatiotemporal variability can represent an important landscape factor in ecological risk characterisation of, *e.g.*, arthropods, plants, aquatic invertebrates. Based on the data underlying the official empirical spray-drift deposition models were developed represented by Probability Density Functions (PDFs). The PDFs can be employed according to the spatiotemporal scales of the landscape-level approach and the aggregation of the local results in the risk characterisation. Temporal variability can be represented by distributions of PPP use sequences and wind directions. The approach is embedded in a conceptual framework of spatiotemporally explicit risk characterisation. The model approach itself might be further developed as more adequate data become available. The software module is Open Source and can be used in different landscape modelling and development environments (*e.g.*, Xplicit, Almass, ArcGIS, QGIS, Python, .NET, OpenMI, SWAT, OpenFluid, *etc*.). Modular design principles can facilitate management of the naturally inherent complexity (scientifically and technically) of landscape-level models, which also enables one to employ specific domain expertise in these multidisciplinary approaches. In combination with professional software integration and maintenance principles, ready-to-use risk assessment 'apps' with official authorisation can be established. Example uses of the approach in landscape-level ecological risk characterisation are given.

WE171

Comparison of methodologies for ecological risk assessment: an application to different chemicals (PFASs, alkylbenzene sulphonate and triclosan) in the river Po, Italy

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Aquatic ecosystem models are useful tools that can improve the ecological realism of chemical risk assessment with respect to the conventional approaches, which are estimating protective ecotoxicological thresholds, such as PNEC (Predicted No-effect Concentration), based on the extrapolation from toxicity data for single species derived in the laboratory to the ecosystem level. However, conventional methods do not fully take the complex effects of ecological interactions into account. Rigorous comparisons of the risk assessed using ecosystem models and through conventional approaches are needed to highlight the advantages and disadvantages of different methodologies, but at the time, there is a lack of such studies. Poly and perfluoroalkyl substances (PFASs) are a large group of emerging contaminants that can be found in the surface water, groundwater and wastewater effluents around the world because of their wide use in the past and nowadays. Considering the persistent nature of some of them (e.g. PFOA) and tendency to be spread over long distances with water, they can be found far downstream of discharge points along watercourses, where they pose risks to human health and aquatic biota. In this work, we carried out a comparative analysis of the ecological risk assessed for selected PFASs in the Po river by using different methodologies. The Po is the longest river in Italy with the greatest discharge, historically influenced by industrial and municipal effluents as it flows through the most industrialized and populated areas of the country. The same comparative analysis was also carried out for two well-known down-the-drain chemicals: the anionic surfactant alkylbenzene sulphonate and the antimicrobial triclosan. Risk was assessed using 1) the risk quotient ratio of PEC (Predicted Environmental Concentration) to PNEC, 2) the statistical method known as Species Sensitivity Distribution (SSD), and 3) integrated ecological and ecotoxicological AQUATOX models. Results show that the three methodologies provide different perspectives on ecological risk assessment and that ecosystem models can lead to unexpected conclusions because they simulate indirect ecological effects that propagate the effect of chemical toxicity across the food web.

Integrated approaches for linking chemical contamination with biological effects (P)

WE172

In situ effects of untreated sewage discharged into the River Danube: expression of selected genes in livers of caged common carp

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In the framework of the EU funded collaborative FP 7 project Solutions, the city of Novi Sad has been selected as the pollution hot spot in the River Danube, mainly due to the direct discharge of untreated sewage into the river. A complex analytical-bioanalytical study [1] based on on-site large volume solid phase extracted water samples has identified severe in vitro biological effects, significantly changed contamination patterns and high concentrations of a number of chemicals in the Danube downstream of the wastewater discharge. Based on the results of the cited study, a fish caging experiment was conducted to check if in vitro observed biological effects can be seen in situ, using a large battery of biomarkers and bioassays in various organs / tissues of caged fish. The fish were exposed in cages for nine days at three sites in the River Danube: upstream (reference site), 700 m and 7 km downstream of the major discharge point of the untreated sewage into the River Danube. Common carp (*Cyprinus carpio*(L.), Cyprinidae),was the species of choice due to its vulnerable status in the Middle Danube, importance in human diet / economic value, favourable traits for caging but also because it is a genetically well described species. The poster will present the first set of data obtained from the study. The liver samples were used for quantitative real-time PCR analysis (qRT-PCR) of selected endocrine disruption and stress representative genes. The expression of six target genes was analyzed: heat shock protein 70(hsp70), cytochrome oxidase subunit 1 (cox1), vitellogenin (vtg), estrogen receptor alpha (erα), estrogen receptor beta(erβ) and cortisol receptor(cr). The differences in expression of selected genes in fish liver from specimens caged at three sites will be evaluated against the chemical composition of water, the results of in vitro bioassays [1] as well as against all other (by the time of the meeting) available results from Novi Sad case study caging experiment.

Reference: [1] König M, Escher BI, Neale PA, Krauss M, Hilscherová K, Novák J, Teodorovic I, Schulze T, Seidensticker S, Hashmi AMK, Ahlheim J, Brack W. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. Environmental Pollution, In Press. *Acknowledgement* – The study is part of the SOLUTIONS project, funded by the EU FP 7 (FP7-ENV-2013-two-stage Collaborative project) under grant agreement number 603437.

WE173

Rapid identification of neuroendocrine-disrupting chemicals in fish using a biomarker - virtual effect-directed analysis (vEDA) approach

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Virtual effect-direct analyses (vEDA) uses multivariate statistics (instead of experimental fractionation) to unravel patterns between data obtained from chemical analyses and bioassays' and it has been recently shown to be effective to unravel contaminants co-varying with mutagenicity in water samples. Virtual EDA is applied here to investigate the accumulation of enviromental toxicants in fish exposed to untreated waste water effluents from urban and industrial sources. Samples used for this study comprise fluids and tissues (incl. blood-serum, liver, muscle) from common carps (*Cyprinus carpium*) collected during a caging experiment carried out in the Danube River (Novi Sad, Serbia) whereas the fish were exposed for 9 days in 4 sites at various pollution degrees. The chosen site had been previously shown as a water pollution hot spot for neurotoxic and/or endocrine-disrupting compounds such as organochlorine and organophosphate pesticides, phthalates, estrogens, neuroactive drugs, etc. The focus of this study is on neuroendocrine-distrupting chemicals (NEDCs), i.e. those that can alter the normal neuroendocrine functions by blocking or mimicking the effects of signaling molecules - neurotransmitters and hormones - and interfering with their synthesis or metabolism, and thus can affect an animal's ability to grow, to reproduce, and to deal with stress or other stimuli. Here, the changes in the levels of a subset of endogenous metabolites (< 50, incl. neurotransmitters, steroid and thyroid hormones) relevant for the neuroendocrine system are used as biomarkers of exposure to NEDCs in fish and vEDA is applied to identify the compounds (or peaks) that co-vary the most with such alterations. Measurements are based on liquid chromatography – high resolution mass spectrometry by using both target and untarget screening. The analytical method has been developed to encompass a wide range of chemical classes in order to allow the determination of both metabolites and environmental contaminants within a certain biofluid/tissue.The biomarker – vEDA approach presented here has the potential to be rapid, sensitive and specific for the detection of NEDCs in fish. Since most of the studies focus on either endogenous or exogenous compounds at a time, there is a scarcity of data on the occurrence of NEDCs and biomarkers in the same biological sample. Thus, their simultaneous determination would benefit a better understanding of the impacts of NEDCs on fish health.

WE174

Physiological biomarkers of an anthropic pollution on a bioturbator key species Limnodrilus hoffmeisteri

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In urban areas, numerous pollutants are accumulated on sealed surfaces (roads, parks, industrialized areas…). …). Rainfall events drained these contaminants, which are finally accumulated in the sediment layer of stormwater infiltration basins. These structures were designed to detoxify runoff water that reached out groundwaters, and it is now necessary to evaluate the toxicity of pollutants in such receptor ecosystems. Only a few organisms - among them the tubificid worm Limnodrilus hoffmeisteri - live in these sediments and play a major key role in the functioning of infiltration basins (e.g. influence of biogeochimical processes, organic matter recycle...). But these organisms could be physiologically impacted by urban pollutants as they are directly exposed (they eat the sediment). The objectives of this study are to measure (i) the oxidative stress and the level of energy body stores (through the concentration of glycogen), (ii) the energetic efficiency of the mitochondria (i.e. the major energy production organite), induced by a multi-pollution in *L. hoffmeisteri* incubated 0, 1, 3 and 6 months in macrocosms containing polluted sediments from different stormwater infiltration basins. Sediments were collected from a blind channel of the Rhône (low polluted sediment) and from three infiltration basins (polluted sediments). At the end of each incubation time, microcosms were dismantled and worms were collected. Then, we measure the level of damage due to the oxidative stress linked to pollution (TBARS) and the glycogen body concentration. The mitochondrial respiration and the ATP production were also measured on a pool of 500 worms. Tubificid worms were clearly impacted by the pollution stress as the TBARS level increase after 3 months of exposure in stormwater sediments, particularly for the most polluted sediments. The glycogen body store also decreased as this compound permit to fuel the costly antioxidant mechanisms induced by pollutants. At the mitochondria level, we observed a decrease in the maximal activity of the electron transport chain (ETC), even if we did not observe a decrease of the mitochondrial efficiency. This result suggested a failure of the ETC induced by urban pollutants.

WE175

Screening of Dutch surface water quality using Daphnia magna bioassays

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According to the European Water Framework Directive (WFD) the chemical status of surface waters is determined by measuring the concentrations of 45 priority substances. However, a large proportion of toxic effects of surface waters cannot be attributed to these 45 priority compounds. Therefore, this project aimed to promote a more effective and relevant method for ecotoxicological water quality assessment by applying bioassays. To this purpose water samples were taken at 28 locations and subjected to acute *D. magna* bioassays (Immobilization test 48h; OECD guideline 202). There were 8 replicates per water sample and 5 *D. magna* per replicate. ISO medium served as control. From samples causing adverse effects on the daphnids, dilution series in ISO medium (2x, 5x, 10x, 15x, 20x, 50x, 100x) were tested. Mobility was assessed after 24 and 48 h of exposure. A general linear model was applied to compare immobilization means of the samples with the corresponding controls. If dose response relationships were obtained in dilution series, EC_{50} values were derived applying a log-logistic response model. 93% of the samples caused no effect on the mobility of *D.magna* and hence no further chemical analyses were required. Only a sample from an Amsterdam harbour and from a greenhouse area caused significant daphnid immobilization after 48h. From the greenhouse area water sample dilution series the EC_{50} value was calculated to be 97.5%. Previous chemical analysis by the responsible waterboard showed that the greenhouse site contained relatively high concentrations of naphtalene, fluorene, anthracene, 44-DDT and 44-DDE. Yet, the concentrations of the measured compounds were still lower than their corresponding EC?? levels reported in literature. Therefore the observed immobility could not be explained by the measured compounds, suggesting the presence of other unknown compounds. The screening of Dutch surface water quality using *Daphnia magna* demonstrated that application of bioassays could avoid expensive chemical analyses, while simultaneously identifying the presence of unknown hazardous compounds that would have been overlooked by routine WFD chemical monitoring.

WE176
Smart Monitoring: Application of innovative tools in nationwide water quality assessment

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The European Union Water Framework Directive (EU-WFD) requires member states to assess chemical water quality of surface waters by monitoring the presence of 45 priority compounds. However, this list is outdated as the selected compounds are not representative for present day contamination. Consequently, a large portion of the observed toxic effects of surface waters cannot be attributed to compounds measured by water authorities. Hence, there is an urgent need for an effect-based monitoring strategy that employs bioassays to identify environmental risk. Therefore, the aim of the present study was to apply innovative tools in a smart, integrated monitoring methodology in a nationwide water quality assessment in The Netherlands. The Smart Monitoring strategy for ecotoxicological water quality assessment applies a combination of passive sampling (PS) with a battery of bioassays to investigate risk to aquatic biota. At 47 locations Silicone Rubbers (SR) and Polar Organic Chemical Integrative Samplers (POCIS) were exposed to surface water for 6 weeks. Alongside the PS a 7-day *in-situ* daphnid test was performed at all locations. Subsequent to field exposure, accumulated compounds were extracted from the PS after which a battery of 3 *in-vivo*, 5 antibiotic SCAN and 10 *in-vitro* CALUX bioassays was exposed to the re-dissolved extracts. The bioassay battery was selected such that it can identify the risk posed by a wide range of chemical pollutants and their transformation products. Bioassay responses were compared to Effect-Based Trigger values (EBT) to identify potential ecological risks at the investigated locations. It is concluded that the smart, integrated monitoring methodology allowed the ranking of sites based on ecological risks, identified the presence of hazardous compounds, regardless of being listed as priority compounds, but meanwhile could prevent costly chemical analysis at sites with low ecological risks.

WE177
Risk drivers in wastewater-impacted streams

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Wastewater treatment plants (WWTP) present a major source of micropollutants to the aquatic environment. Aquatic organisms are therefore constantly exposed to chemical mixtures, which can impose negative impacts on the ecosystem. In this study, we investigated concentration patterns of a large number of micropollutants in wastewater-impacted streams, as well as in the effluents, across independent catchments with different land uses. Acute risk was predicted using the multi-substance potentially affected fraction approach and compared with biomonitoring data using the SPEAR index. Grab samples were taken at 24 Swiss WWTPs (effluent, upstream, downstream) during eight time points and analyzed for almost 400 organic substances. Besides pharmaceuticals and other typical household chemicals, also many pesticides were included, as we wanted to investigate whether the higher loaded pharmaceuticals or the episodically discharged pesticides - when released during low flow conditions - contribute most to the risk towards aquatic organisms. Macroinvertebrate data was collected at the same sites during two time points in spring. As expected, a multitude of micropollutants was regularly detected and the concentrations were mostly higher downstream than upstream. Further, a positive correlation of plant protection products upstream and arable land use could be observed. While pharmaceuticals and other typical household chemicals were regularly detected in the effluents, many pesticides were detected only during episodic events and are thus underrepresented with grab samples. Nevertheless, occasional concentration peaks were observed for pesticides in the stream as well as in the effluents and the acute toxic pressure was mainly driven by pesticides. The lack of effect data for pharmaceuticals limits, however, interpretation for this substance group. Overall, rather low acute risk was predicted ranging from 0% to 2.1% of affected species over all sites and time points with only a few substances explaining already the total risk. Despite the low predicted risk a significant positive correlation with the SPEAR index was observed, highlighting the importance of pesticides in wastewater-impacted streams. Currently determined internal concentrations of micropollutants in macroinvertebrates, specifically in gammarids, collected at the same sites will give even more insight into the relevant substances contributing to the biological effects observed in the field.

WE178
Skylarks nesting in pesticide-treated crop fields compared to those nesting in non-treated agricultural land and main reasons for complete nest loss

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Pesticides are commonly mentioned to be a cause of nest failure or species decline. However, data on pesticide use (e.g. over-sprayed nests) and subsequent monitoring of such nests are virtually lacking. Studies about species decrease mention pesticide as likely reason, or focus on detectable reasons (predation, flooding etc.) with no information about the pesticides used at the study site. Over the period 2007 – 2012, the nests of 83 skylarks breeding in either pesticide-treated arable crop fields (winter wheat, spring cereals or oilseed rape) or non-treated grassland habitats (organic, conventional or alfalfa) were located and observed. In each case, the development of the brood was documented and various brood parameters recorded until the nest was either lost or the chicks had fledged and breeding success could be confirmed. The use of pesticides (pesticide type, active ingredient, concentration and number of applications) was recorded for each of the treated fields harbouring skylark nests. Up to eight pesticide applications were made to the treated fields during the breeding period of specific nests. Although potential effects of pesticide use on breeding success cannot be classified, predation was found to be the main cause of nest failure in the treated crop fields, and in untreated grassland habitats, the majority of nest losses (and overall higher) were due to mowing activities carried out on these areas.

Risk assessment and remediation of mine sites and processing sites (P)

WE179
Metal cycling in constructed wetlands receiving highly alkaline steel slag leachate

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The use of wetlands for the treatment of metal-rich waters is usually cautioned against where there is risk for significant biological cycling of metals and potential uptake to higher organisms. This could be the case under alkaline conditions where the presence of oxyanion-forming elements can mimic essential plant nutrients (e.g. vanadate behaves similarly to phosphate). This study reports on analysis of waters, sediments and plant material in constructed wetlands receiving hyperalkaline (pH < 13) steel slag leachate. Steel slag waters are dominated by Na-OH type waters due to the presence of Na-rich desulphurisation slag in the disposal heaps at the study site in northern England. The leachates are also enriched in vanadium (V: up to 250 ppb), while the treatment wetland sediments, characterised by extensive secondary calcite deposits as a product of leachate buffering, have roughly double the V concentrations (mean of 230 ppm) of nearby reference sites receiving uncontaminated groundwater and surface runoff (mean of 100 ppm). Significantly higher V was also apparent in rhizome material of *Phragmites australis* in the treatment wetlands than reference sites, although most other metals of concern (e.g. Cd, Cr, Mo, Pb) did not vary significantly between sites. Encouragingly, there no evidence of translocation of V to aboveground portions of the plant where risks for uptake by grazers could be anticipated. The implications for adoption of wetlands to manage highly alkaline steel slag leachates are discussed.

WE180
Exposure-based risk assessment of historic aluminium smelters in France to inform continued water monitoring responsibilities

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Limited; A. Boullemant, Rio Tinto / Lergacy Management
Pollution prevention and control is an integral component of industrial operating permits awarded by the local authority in many jurisdictions, and is also mandated under the EU Industrial Emissions Directive (2010/75/EU). Pollution management is informed by emissions monitoring, of which is an ongoing practise, even after site closure. Termination of monitoring is recommended if it can be adequately demonstrated to the local regulatory authority that there are no potential environmental or human health risks posed by the site to the local environment. This poster presents a methodology for exposure-based risk assessment and discusses recommendations to the water monitoring programme that can be made from the assessment. We analysed water monitoring data from 11 legacy aluminium processing and waste disposal sites in France to identify sites which pose no ecological risk to the surrounding environment, and therefore no longer require monitoring. We compared the data to national and international environmental quality standards (EQSs) for drinking water (e.g. as established by France, the EU Water Framework Directive and the World Health Organisation) using a tiered approach. A high-level screening was completed, considering data from all monitoring timepoints (up to 20 years) to identify risk chemicals, followed by verification of risk chemicals using the last 5 years of data. Statistical analysis was also conducted to understand the frequency of future exposure exceedances. Sites which do not pose any potential risks to the local environment are identified, and recommendations are made for future monitoring activities at sites which cannot currently be identified as posing no risk.

WE181

Ecological impacts and ecosystem response in an old mining area of SE Spain: learning lessons for phytomanagement

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This presentation shows the results of a study in which field and greenhouse works were performed in an old mining area (Cartagena-La Unión SE Spain). The objective was to understand the plant colonisation mechanisms in sites affected by mine wastes (tailings and polluted salt marshes) to take advantage of the natural processes for phytomanagement prurposes. Field works studied the environmental gradients, soil-plant relationships, plant-favourable edaphic niches, key species, seed bank and metal(loid) accumulation in soils and plants. Greenhouse works gave informaton about the effects of soil amendments (municipal waste refuse -MWR-, biochar -B- and liming -L-) on metal(loid) geochemistry and plant growth. Vegetation colonisation was favoured by the existence of edaphic niches with favourable conditions; soil salinity played a key role. In the mine tailings patches with sandy texture and lower salinity showed the most favourable conditions for pioner vegetation (grasses and shrubs), improving the nutritional and microbiological status of rhizospheric soils, while those more saline and with fine texture showed no plants. Late successional stages with climax vegetation were found as part of the so called fertility islands, with a key role of trees. In the salt marsh the most saline and wettest sites were the most affected by the mine wastes, showing no vegetation. Plant colonisation of mine tailing bare soils was strongly limited by the absence of seeds, while in the salt marsh by the absence of germination of the present seeds. Plants accumulated more metal(loid)s in belowground tissues. Greenhouse experiments showed that in the mine tailings MWR improved soil fertility and plant growth, but enhance metal mobility, while B increased water holding capacity and reduced metal mobility, but was less effective than MWR providing nutrients. Under hydric conditions (salt marsh) B and L amendments favoured decreasing Eh values, increasing metal mobility, especially in the presence of vegetation. The use of specific remediation measures is strongly advised in mine tailings and sites polluted by mine wastes. The presence of diverse plant species, with contrasting strategies to cope with multiple stresses, may result in a more efficient employment of the water resources and a higher biodiversity. The effectiveness of different amendments (organic and inorganic) may be influenced by the soil characteristics but also by the biogeochemical conditions of the target site.

WE182

Contribution of active biomonitoring in environmental risk assessment on a former mining site

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Past industrial activities have contaminated the environment with trace metals (TMs) and we have now to evaluate and to limit the impact of this pollution. Biological methods using bioindicators of accumulation to assess *in situ* the fate of TM are scarce. On the former mine of Saint Laurent le Minier (30, France) soils are highly contaminated by arsenic, cadmium, lead and zinc (mainly). To evaluate current exposure of soil organisms to metal pollution, a biological indice have been developed based on the determination of the Sum of the Excess of Transfer of metals (SET indice) from soil, plants (growing on this soil) to the common land snail *Cantareus aspersus*. Calculation of the SET indice is simple: SET_{plot}= Σ(AQ-1), with AQ (accumulation quotient for each metal = [Concentration in snails caged for 28 days on the site]/ [Internal concentration of reference: CIRef] (https://ecobiosoil.univ-rennes1.fr/ADEME-Bioindicateur/english/worksheet.php). The aim was to rank the management priorities of the 8 plots of the site based no longer on the total TMs concentrations in soil but on the metal bioavailability (i.e. on the excess of transfer) of 14 TM to land snails caged for 28 days on site. Results showed abnormal transfer on all plots except on the control one. The highest transfers were found for Cd, Pb, Tl and Zn. For the first time extremely high internal concentration of thallium were evidenced (up to 2639 $mg.kg^{-1}$, 3595 higher than the Tl-CIRef value) in the snail viscera. SET_{plots} were from 57 to 3876 and the SET_{site} was 924 based on the analysis of 14 TMs. Previous studies using the SET indice only reached SET_{site} of 7.7, showing the very high bioavailability of TM on the studied site. Comparison of the SET_{plot} indices allows ranking the plots according to the biological assessment of bioavailability of TMs; the biological ranking does not match with the chemical ranking based on total concentration of TM in soils. For example, the soil of one of the most contaminated plot presents a transfer close to the transfer determined on the less contaminated plots due to a reduce bioavailability. Present results show how bioindicators of accumulation, that integrate *in situ* the influence of various environmental parameters (soil characteristics, plant contamination, season), can contribute to the assessment of fate of pollutants in the field and also to the development of a management plan adapted to the context of the polluted site.

WE183

DETERMINATION OF POLLUTION LEVELS OF SOME HEAVY METALS IN THE COAL MINING ENVIRONMENT OF UDI, AKWUKE AND NGWO COMMUNITIES OF ENUGU STATE, NIGERIA.

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SOIL, WATER AND FOOD CULTIVARS IN THE COAL MINING COMMUNITIES IN ENUGU STATE WERE INVESTIGATED FOR THE PRESENCE OF HEAVY METALS IN THE SOIL, WATER AND FOOD CULTIVARS. THREE OF THE COMMUNITIES ARE COAL MINING IMPACTED AREAS, THOUGH COAL MINING HAS BEEN STOPED FOR OVER 15YEARS NOW IN THESE COMMUNITIES. THE HEAVY METAL OF SOIL, WATER AND FOOD CULTIVARS FROM THESE AREAS WERE INVESTIGATED BY ATOMIC ABSORPTION SPECTROPHOTOMETER. THE RESULT OF HEAVY METAL LEVELS IN SOIL OF THE THREE COMMUNITIES ARE WITHIN THE PERMISSIBLE LIMIT IN AGRICULTURAL SOIL (AWASHTHI,2000), THOUGH HIGHER LEVEL OF Mn, Pb, AND Cd WERE OBSEREVED IN AKWUKE SOIL OVER OTHER COMMUNITIES WHICH COULD BE ATTRIBUTED BE ATTRIBUTED TO ANTHROPOGENIC AND MINING ACTIVITIES THAT TOOK PLACE IN THE AREA, AND USE OF FERTILIZER. THE HEAVY METAL LEVELS IN WELL WATER FOLLOWED THE SAME TREND AS OBSERVED IN SOIL. HOW EVER Mn, Cr AND Pb LEVEL IN WELL WATER FROM THE THREE COMMUNITIES WERE ABOVE W.H.O (2005) PERMISSIBLE LIMIT OF 0.05mg/l, 0.05mg/l, and 0.01 FOR Mn, Cr, AND Pb RESPECTIVELY. THE HIGH LEVEL OF THESE METALS IN WATER COULD BE ATTRIBUTED TO ANTHROPOGENIC SOURCE AS WELL AS UNDERGROUND WATER POLLUTION DUE TO LEACHING OF THESE METALS IN THE SOIL TO UNDERGROUND WATER. FOOD CULTIVARS FROM THE THREE LOCATIONS WERE EQUALLY PROPORTIONATELY CONTAMINATED WITH THESE HEAVY METALS. THE LEAFY VEGETABLE (PUMPKIN AND SCENT LEAF) WERE FAR MORE CONTAMINATED THAN THE TUBERS (YAM AND CASSAVA). THIS RESULT REVEALS THAT COAL MINING ACTIVITY IS RESPONSIBLE FOR PREPONDERANCE OF HEAVY METALS IN THE WATER OF ENUGU STATE. IT IS SUGGESTED THAT THE WATER AND FOODS REMAIN MAJOR ENDOGENOUS SOURCE OF HEAVY METALS AMONG THE PEOPLE OF THE AREAS.

WE184

Long term coal mining activities in Enugu metropolis; could it be responsible for bioavailability of heavy metals in plants?

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Coal mining activities which spanned over a period of 48 year left several mine pits and tunnels in Enugu metropolis. Since activities stopped in 2005, proper site closure was not put in place. Already identified critical problem created is the pollution of potable water supplies as a result of the acid mine drainage from abandoned coal mining pits. Since site-specific characteristics have a major influence on contaminant bioavailability, we examined the possible effect of coal particulates on bioavailability of metals and hence uptake by plants. Soil and water samples, as well as plants/crops cultivated in three communities, Ngwo, Akwuke and Udi, in Enugu coal mining areas were analysed for heavy metals levels. Parallel control experiments were run using similar soil samples devoid of coal particulates, but spiked with different concentrations of heavy metals. The samples used in this study are yam (*Dioscorea rotundata*), c*assava (Manihot esculenta),* scent leaf

(*Ocimium grattisimium),* fluted pumpkin leaf (*Telifaira occidentalis*), and soil. Plant materials and soil samples were analysed for heavy metals using Atomic Absorption Spectrophotometer (AAS) according to American Public Health Association (APHA). Bioaccumulation factors (BAFs) for lead, chromium, nickel, cadmium and manganese in plants were determined. Also the transfer factors of these metals from soil to crops were determined. Results suggest that coal particulates tend to play some significant role in some metals uptake by some tubers and plant leaves. The transfer factors of these metals for some plant cultivars lay credence to this view. Results obtained also suggest that high concentrations of toxic metals in soil within Enugu metropolis could have accumulated due to about 48 years of coal mining activities, resulting in their transfer to food chain.; a threat of phytotoxicity to the local population. This calls for more robust site-specific investigation to guide the government in making informed decision and policy formulation for the protection of Enugu community.

Toxicology and Ecotoxicology, human and ecological risk assessment of engineered nanomaterials: needs, goals and tools/methods for safer-by-design strategies (P)

WE185

High dimension biological analysis of carbon nanotube toxicity

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The toxic effects of carbon nanotubes (CNTs) have been studied for several years and adverse responses have been identified in several tissues, including cytotoxicity in keratinocyte cells and inflammatory and fibrogenic responces in pulmonary tissues. It has been also found that CNTs produce a time- and dose-dependent toxic response upon reaching the lungs in sufficient quantity. Beyond time and dose, chemical functionalization affecting water solubility, dispersibility and agglomeration tendency, as well as impurities, amorphous carbon, surface charge, shape, length, and layer numbers have been recognized as significant modulators of toxicity. The current study aimed at identifying the effects of exposure to SWCNTs (single-walled carbon nanotubes) and MWCNTs (multi-walled carbon nanotubes) of different functionalisation at different levels of biological organization, and at shedding light on the potential mechanism(s) of action. For this purpose, (a) immunological, (b) biochemical, (c) gene expression and (d) biological pathway analyses were carried out, combining human biosamples and in vitro testing, focusing on the mechanisms through which CNT exposure induces immunological responses. The study involved 12 healthy volunteers with an age range from 25 to 30 years (mean age 27±2.1). Venous blood samples were collected in the early morning following overnight fasting in vacuum EDTA tubes (Vacutainer). The doses of WNTs were selected according to the recommended exposure level of 1 $\mu g/m^3$, according to NIOSH. The MWCNTs with more complex structure caused a significant increase in lipid hydroperoxides levels and in the percentage of immune cells with reduced mitochondrial membrane potential as compared to the simpler CNTs. Analysis of the effects of different types of MWCNTs on gene expression showed that impurities influence significantly the induction of key toxicity pathways such as inflammation mediated by chemokine and cytokine signaling. Pathway analysis showed significant modulation of genes related to the NFkB pathway, after exposure to more complex MWCNTs, as a result of oxidative stress induction. This may cause a perturbation of the IL-6 pathway that aims to regulate the inflammatory processes and compensate apoptotic changes. Overall the immunological responses related to CNTs exposure are considered the result of the synergistic effect of systemic (mediated by cells of the exposure routes) and local inflammation (blood cells).

WE186

Proposal for a framework to calculate the environmental, safety and Health impacts of nanofertilizers

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The use of agrochemicals is crucial to modern agriculture.In fact, fertilization practices increases crop efficiency and lead to a better quality of product recovery in agricultural activities. However, fertilizer consumption has increased exponentially throughout the world in the recent years and it has causedserious environmental problems, such as water, soil and air pollution. Because of these reasons, the reduction in chemicals useand their replacewith environmentally-friendly compounds has emerged as ones of the most important R&D needs. In this regard,nanotechnology has the potential to revolutionize the traditional agricultural practices.Nanomaterials(NMs)can both decrease the use of applied fertiliser andincrease their efficiency on crops. Nevertheless, one of the most common compounds used in those products is nanoZnOwhich has showed adverse effects towards different soilorganisms. The aim of the study is to investigate the environmental and human health impacts of the use of nanofertilizers withnanoZnOcompound. The study will start with the definition of the ecotoxicological parameters (NOEC, EC50) for NMs released from fertilizersin soil matrices and soil lecheates, upon non-target organisms (eg. microbial communities, earthworms etc.), which are necessary to understand the impact of nanofertilizers. Moreover, the risk upon human health associated with the emission during synthesis, manufacturing and application of these nano-agrochemicals should be evaluated, by defining the NOAEL and LC50 in each phase of the exposure.Finally, Life Cycle Assessment methodology, which is a standardised tool recognized at international level for quantifying and evaluating the potential environmental impacts of productsthroughout their life cycle, will be applied to eassess the environmental performance of nano-fertilizerswith nanoZnO compound from their production until the use phase on the soil. In particular,the models for the calculation of emissions into air, water and soil from the use of nano-fertilizers will be taken into account. This integrated research strategy has the objective to highlight the possible trade-offs between the increase in fertilizer efficiency in terms of lower consumption in the soil and bothecotoxicological and human risks.

WE187

NanoCRED: A transparent framework to assess the regulatory adequacy of ecotoxicity data for nanomaterials - relevance and reliability revisited

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Environmental hazard and risk assessment is the foundation for regulatory decisions to protect the environment from unintended adverse effects caused by chemical substances including nanomaterials. The risk assessment process requires relevant and reliable environmental hazard data upon which Predicted No-Effect Concentration (PNEC) values can be estimated - and upon which classification and labelling can be based. In a regulatory context ecotoxicological data is often considered more valid for regulatory use if obtained according to accepted and validated test guidelines, preferably also following Good Laboratory Practice (GLP). It is known, however, that engineered nanomaterials behave very differently in ecotoxicity tests when compared to the 'conventional' soluble chemicals, for which most test guidelines were developed. Therefore non-standard tests, or tests following modified test guidelines, can provide valuable information on nanomaterial hazards and should not *per se* be considered less reliable. To support expert judgement and facilitate a transparent evaluation of available ecotoxicity data for nanomaterials, we propose twenty criteria for evaluation of ecotoxicity data reliability. These criteria take into account the testing challenges and characterisation requirements associated with nanomaterial ecotoxicity testing. The criteria were developed to be used in combination with the method developed through the 'Criteria for Reporting and Evaluating Ecotoxicity Data (CRED)' project. Combining criteria for evaluation data relevance with criteria for data reliability an overall evaluation of data adequacy can be made. This approach was developed to accommodate all types of nanomaterials, all types of aquatic ecotoxicity studies and support qualitative as well as quantitative data evaluation requirements. Furthermore, it is intended to be practically feasible to implement and directly applicable in European as well as international regulatory frameworks.

WE188

Grouping of nanomaterials regarding their ecotoxicity - are hypotheses based on literature data robust enough?

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Nanomaterials (NM) offer a great innovation potential and are assumed to be beneficial to mankind and the environment by e.g. reducing material usage and energy consumption. However, the large variety of synthetic nanomaterials represents a major challenge for scientists and regulators in terms of measuring and assessing the potential hazard caused by the materials and the products over the whole life-cycle. Currently, the assessment of potential hazards posed by NM towards environmental organisms is assessed on a case-by-case basis, which is considered as not practicable to be performed for the many different variations of NM, which involve differences in composition, size, shape, crystalline structure and surface modifications. In order to overcome the need for extensive testing grouping and read across approaches for NM are discussed, enabling risk assessors to predict the hazard of an NM based on existing knowledge on similar NMs. This requires the identification of relationships between nanomaterials' physicochemical properties and their ecotoxicological behavior. To gain knowledge on this relationship, we performed an extensive literature research taking into account ecotoxicological studies either involving several organisms and / or several modifications of the same type of NM. This allowed us to reveal parameters crucial for the emergence of specific ecotoxicological effects. The set of parameters identified as relevant included the surface properties (charge, zeta potential, surface modifications), the size and shape of a NM, and the release of ions. Based on these findings, a test set of NM, involving in most cases several subtypes of a given NM was compiled, which underwent testing in the ecotoxicological relevant aquatic (algae, daphnia and zebrafish embryo) and terrestrial organisms (earth worm, microorganisms). Subsequently, the initial grouping hypotheses were compared to the actual results obtained in the tests in order to verify or falsify initial grouping hypotheses. Even though several predictions could not be confirmed, a first evaluation of results shows that inert and ion-releasing NM needs to be considered differently. *Acknowledgement* - Funding of the project nanoGRAVUR by the German Federal Ministry of Education and Research (BMBF), Grant No. 03XP0002 is acknowledged. **Keywords**: grouping, read across, toxicity profiles

WE189

Grouping of nanomaterials regarding aquatic ecotoxicity - hypotheses for selected NMs and experimental proof

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Due to the large variability of nanomaterials (NMs) the information gathering process needs to be efficient and read-across and category approaches are a pragmatic way to reduce the amount of testing and to characterise the hazards. So far, various parameters relevant for the grouping of NMs are discussed, but they are not specified, combined, ranked, and no further decisions are drawn yet. To promote the discussion and to support the implementation we applied a systematic testing regime and identified some relationships between ecotoxicity and NM-properties. Ion-releasing and inert NMs such as Ag, ZnO, CeO_2 and TiO_2 were included. Several forms per NM-type were considered differing mainly in size, shape, crystalline structure, surface area, solubility and zeta-potential. Specific surface modifications where excluded. Aquatic ecotox-tests with algae, daphnids and fish embryos according to the OECD test guidelines 201, 202 and 236 adapted for the testing of NMs were performed. NM properties influenced by the medium composition were determined in the test media. It was obvious that mono-causal relationships do not exist. For ion-releasing NMs the solubility is discussed as relevant parameter. However, due to significant discrepancies between solubility and ecotoxicity, we propose that for ion-releasing NMs mechanical and morphological properties as well as the test organism have to be considered. Solubility as single basis for grouping and read-across seems to be suitable only in certain cases. The crystal structure is also discussed as relevant parameter regarding ecotoxicity. This seems to be of particular relevance for inert NMs such as TiO_2 and CeO_2. Doping of NMs with a small amount of additional non-toxic elements can modify the structure. Although this modification was not obvious with measurements such as ROS activity and CPH reactivity, toxicity was modified by a factor of about 10. For CeO_2 additional parameters such as surface area seem to be of relevance. From the results a rough grouping approach in ion-releasing and inert NMs seems to be justified. There are indications that ion-releasing NMs are more toxic than inert NMs, whereby the material specific toxicity of the ion-releasing NMs has to be considered. A simple grouping of NMs (same chemical composition) or read-across considering just one parameter does not seem to be possible. Various physico-chemical parameters and the dependence of data on the test organisms have to be considered.

WE190

Does encapsulation of the biocide DCOIT in silica nanocontainers reduces its toxicity? Effects on the photophysiology and cell cycle of the endosymbiotic algae Symbiodinium sp.

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The fouling of biomolecules and organisms onto aquatic surfaces is responsible for undesirable species dispersion, surface corrosion, and increases fuel consumption of ships. Use of the antifouling biocide DCOIT (4,5-Dichloro-2-octyl-2H-isothiazol-3-one) is considered an environmentally acceptable method of preventing marine species attachment (European Directive 98/8/EC), however, information regarding its toxicity to non-target species is scarce. With advances in nanoparticle engineering, the encapsulation of biocides in nanocontainers as smart-releasing systems has received increased attention. The present work aimed to access the impacts of a new antifouling approach, DCOIT encapsulated in silica nanocontainers (SiNC@DCOIT), toward the non-target species *Symbiodinium* spp., the endosymbiotic algae of reff-buiding corals and other marine invertebrates. Elevated seawater temperatures, as predicted under global climate change scenarios, are known to have strong negative effects on *Symbiodinium* physiology. DCOIT is also a known inhibitor of photosynthesis, and thus there is the potential for a joint effect of the two stressors. Toxicity assays were performed by exposing *Symbiodinium* strains Ap31 and 2464 to a range of 5 concentrations (15 – 240 $\mu g\ L^{-1}$) of empty silica nanocontainers (SiNC); SiNC@DCOIT and DCOIT at two different temperatures (26 and 30.5°C) for 72h. Photosynthetic parameters (F_v/F_m; ETR_{max}; α; and *NPQ*) were characterized using a rapid light curve protocol on a Pulse Amplitude Modulated fluorometer (PAM). The effects on cell cycle dynamics and ROS production were monitored by flow cytometry, using the probes DAPI and DCFH-DA respectively, while growth inhibition was access according to the OCDE 201 guideline. After 24h, maximum quantum efficiency of photosystem II (F_v/F_m) reduction of 20% was observed at 30 $\mu g\ L^{-1}$ or 60 $\mu g\ L^{-1}$ DCOIT in 2464 and Ap31 strains respectively, but recovery capacity was evident, with F_v/F_m returning to baseline levels after 72h exposure. Further, 50% growth inhibition rate (%Ir) was evident at 12.5 $\mu g\ L^{-1}$, (DCOIT) compared to SiNC@DCOIT (50% Ir at 23 $\mu g\ L^{-1}$), showed strong DCOIT toxicity. The SiNC present had no effects at lower concentrations, but shows 50% Ir above 60 $\mu g\ L^{-1}$. The growth effects can also be accessed in terms of cell cycle progression and ROS levels, improving our capacity to address toxicological responses at population level. **Keywords:** Nanotechnology; Photophysiology; Global changes; ROS

WE191

NANOGRA: TiO2, CNT and Al nanoparticles risk assessment for sediment-dwelling organisms

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The increasing use of nanoparticles for manufacturing numerous and varied materials and products raises questions about the risks they may represent for both the public and industries that use them. As such, nanotechnologies and their products represent a serious challenge to the authorities responsible for environmental policies and human safety. The NANOGRA project proposes a global approach to nanoparticle's risks using a multidisciplinary assessment based on different fields of study: - The explosion and inflammation risk of the nano-sized particles by determining different parameters of interest - The potential impacts to ecosystems associated with a certain dispersion of nanoparticles in environmental media assessed by laboratory experiments before extrapolating to field experiments. - The toxicological risks for exposed workers and consumers through the use of some product lines are evaluated using several graded risk management tools ("control banding"). A comparative study of the various management tools is also conducted on selected nanoparticles for the two previous components. This presentation focusses on ecotoxicological aspects of NANOGRA. In the case of an accidental release of nanoparticles in the environment in Belgium, sediments will likely be the final sink for the released nanoparticles (leaching of dust for ex). Sediment organisms will probably be the most affected by this new kind of contaminants. A first laboratory approach on artificial sediment spiked with nanoparticles should assess the lethal and sublethal risk for *Chironomus riparius* (bioaccumulation, growth inhibition, development delay and teratogenicity) and *Heterocypris incongruens* (growth inhibition). The nanoparticles were selected on the basis of different nanoparticles emission sources identified in Wallonia and/or industrial strategic directions. Three nanoparticles have been chosen: titanium dioxide, multiwall carbon nanotubes and aluminium. The experimental protocols will be presented and the first available results will be discussed.

WE192

Effects of coating agents on ecotoxicity of silver nanoparticles

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Development of nanotechnology is adding new kinds of nanoparticles with different properties to the environment. Surface properties of nanoparticles play an important role in determining toxicity of nanoparticles (NPs). However, effects of surface properties of NPs on toxicity are poorly understood and cannot be generalized to determine the risks. Surface properties of NPs and the interface domain between NPs and surrounding environment influence physico-chemical characteristics and behaviour of NPs in the aquatic environment. These changes plus intrinsic properties of NPs and organisms affect the level of exposure, biological uptake, and lethal and sub-lethal effects on organisms. We followed an interdisciplinary and multianalytical approach to investigate the effects of silver NPs coated with three different ligands; Tyrosine (T-AgNP), Epigallocatechin gallate (E-AgNP) and Curcumin (C-AgNP) in relation to the toxicity to a key aquatic organism; *Daphnia carinata*. The study focussed on how coatings determine fate of NPs after suspension in the media, lethal and sub-lethal effects, bio concentration and trophic transfer from unicellular alga, *Pseudokirchneriella subcapitata* to daphnids. NP stability tests indicated that T-AgNPs are least stable with increased ionic strength of media, but show the least toxicity to daphnia based on 48-h LC_{50} values while E-AgNP showed highest toxicity. Trophic transfer studies showed that the bioconcentration of AgNPs in algae and the bioaccumulation of NPs in daphnia from algae vary for differently coated AgNPs. Highest retention percentage of silver (Ag) in daphnia from trophic transfer from algae was observed for T-AgNPs followed by E-AgNPs and C-AgNPs. The knowledge generated from this study enhances the understanding of surface property dependent effects of NPs to aquatic organisms. Key words: silver nanoparticles, ecotoxicity, coating, daphnia

WE193

Safe-by-design in practice: Met@Link project

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Safe-by-design principles are applied in the Met@link project. New types of printing inks for tags are developed in this project. The inks are metal-based and enriched with Ag-nanoparticles (Ag-NP) for improved technical performance. Next to economic arguments, and requirements on efficiency and technical performance, also the safety of the newly designed inks and the final product for man and environment is taken into account at the different steps of product development. Literature on Ag-NP learns that these particles (and Ag-ions) are very toxic to aquatic life Two prototype inks were formulated so far. These inks are not soluble in water, but leaching should be taken into account as a potential exposure scenario for these materials. Both inks were tested for their leaching potential: 1g of each ink was suspended in 1l demineralised water and stirred for 24 hours at room temperature. After removal of the solids by filtration (45 μm), the aquatic fraction was used for testing. Ag-concentrations (ICP-AES) and Ag-NP (SP-ICP-MS) were measured, and the ecotoxic effects on algae (OECD 201) and *Daphnia* (OECD 202) were measured. The testing of the varnish (ink without Ag-NP) is ongoing. Both prototypes showed significant leaching behaviour: 3640 and 2610 μg/g of Ag was released from the inks in the 24 hours leaching test. Nanoparticles (>25-30 nm) however could not be detected above detection limit, indicating that Ag-ions are released from the Ag-NP. As expected from the Ag concentration, both leachates were highly toxic to *Daphnia* (EC_{50} values were respectively 0.037% and 0.033% leachate for the 2 prototypes) and algae (EC_{50} values were 0.15% and 0.23% leachate for the 2 prototypes).These EC_{50} values are equivalent to 1.35 and 0.861 μg/l Ag (*Daphni*a) and to 5.46 μg/l and 6 μg/l Ag (Algae) which are lower than reported literature data for Ag-ions. Results show that the current prototype inks both are a potential risk for aquatic organisms when in contact with water (waste water). The incorporated Ag-NP release Ag- ions to the aquatic fraction up to highly ecotoxic levels. This has to be taken into account in further product development f.i. by using Ag-NP with lower leaching potential, develop a matrix that prevents release of Ag, or provide solutions to recollect the Ag-ions from waste water.

WE194

The influence of media components on the observed antibacterial effect of silver ions on Bacillus subtilis - a different approach to research silver toxicity

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Silver is a noble metal which has been used for centuries due to its effective antibacterial, antifungal and antiviral properties. For long, silver and silver salts are used for preserving water or food and preventing infections of wounds. More recently, silver nanoparticles (AgNPs) were developed, resulting in a remarkable increase in silver usage. In order to predict the possible ecological and human toxicity of silver upon release into the environment, as well as to verify the antimicrobial effect of new products and applications, a large number of scientific papers describe toxicity tests of AgNPs, silver ions (Ag^+) and colloidal silver towards environmentally and clinically relevant bacteria, both in growth media or biological matrices. However, contradictory results are reported with inhibition concentrations varying by a 100-fold. We investigated whether this variance in results could be attributed to the difference in testing conditions, especially the microbial growth medium. In this study, *Bacillus subtilis* was exposed to 500 μg/L Ag^+ - which is the main active species of silver - in growth media with different concentrations of some commonly used media components. The toxic effect was investigated by flow cytometry, transmission electron microscopy and by analyzing the growth curves obtained by optical density measurements. The results showed a strong influence of media components on the observed toxicity of silver. Therefore, researchers have to be aware of the possible interference of media components with Ag^+, and should consider their influence before drawing conclusion.

WE195

Chlorophyll fluorescence of the aquatic macrophyte Spirodela polyrhiza for rapid response of exposure to toxicants

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The rapid response of the aquatic macrophyte, *Spirodela polyrhiza*, to toxicants was studied by examining chlorophyll a fluorescence using a Junior-PAM (Pulse Amplitude Modulated). *S. polyrhiza* plants were exposed to two different types of toxicants (antimony and 2,4-Dichlorophenoxyacetic acid) for 1 hr and maximal fluorescence of light-adapted plants was then measured. In response to varying concentrations of 0, 0.045 0.09 0.18, 0.36 and 0.72 mg L^{-1} of antimony, values of Y(NPQ) showed 0.509, 0.505, 0.448, 0.478, 0.319 and 0.373, respectively. Y(NPQ) values were found to 0.486, 0.500, 0.431, 0.396, 0.432 and 0.399 after exposure to 2,4-D with varying concentrations of 0, 0.075, 0.15, 0.3, 0.6, and 1.2mg L^{-1}, respectively. With the national drinking water standards of antimony and 2,4-D being 0.18 mg L^{-1} and 0.3 mg L^{-1} in 1 h in mind, the present results revealed that there was a clear decrease in Y(NPQ) by 6.09% and by 18.52% after exposure to antimony and 2, 4-D of allowable levels for natural drinking water, respectively as compared with the controls. The use of Chl *a*-fluorescence may be a useful biomonitor to assess toxicity in drinking waters within 1 h.

WE196

Needs and challenges for developing an Integrated (eco)toxicological Risk Assessment of engineered nanomaterials

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Nanotechnology represents an important technological option, useful to improve sustainability. It is critical, however, to properly define the needed conditions so that nanomaterials (NM) and related productions could be sustainable from the design and for the entire life cycle. To this aim it is essential to perform Environmental Risk Assessnment (ERA) and Human Health Risk Assessment (HHRA). For historical and practical reasons, ERA and HHRA have generally developed independently using different terminologies and largely separate data, models and assumptions. But despite such unavoidable differences, ERA and HHRA also overlap in several instances: fate models are essentially based on properties of environmental compartments (soil, plants, etc) and on common properties of chemicals (e.g. partitioning and degradation in environmental media). Moreover, species that are assessed in the frame of ERA can also form part of the human food chain (e.g. fish), etc. The concept of integrated risk assessment (IRA) has been proposed as a potential solution because it brings together independent sources of (eco)toxicity data to enable a more harmonized, comprehensive, informative and efficient risk analysis process. In the current industrial climate, the importance of effective “nano” IRA could be paramount, not only to help identify and quantify the potential adverse impacts of nanomaterials to humans and the environment, but also to help industry develop ‘green’ products and applications with the best knowledge available. Data reliability and relevance are two key components for successful IRA implementation. To improve ecotoxicology quality of Engineered Nanomaterials (ENMs), it is necessary to perform study with more ecological relevance and environmental realism. Similarly, for human toxicity relevant data, studies should aim to describe adverse outcome pathways from initial intake of nanomaterials, through molecular, cellular and physiological alterations, to possible ultimate health impact. As a contribution to the development of ENM IRA, we will discuss some needed criteria for ENM (eco)toxicity integrated hazard characterization, taking into consideration the entire material life cycle.

WE197

Human Health and Ecological Risk Assessment and Management of nano-enabled products through the life-cycle

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While the global nanotechnology value chain is expected to reach $4.4 trillion by 2018, and nanosafety research funding has been on a rise since 2005, large uncertainties about the environmental, health and safety (EHS) risks of nanomaterials (NM), including physicochemical characterization, environmental release, exposure and hazard estimation and risk characterisation, continue to persist. In order to address these emerging issues, European Chemicals Agency (ECHA) has adopted a bottom-up approach in REACH implementation through two working groups (i.e. NanoMaterials Working Group and Group Assessing Already Registered Nanomaterials). The registration of the nano-form of chemicals has been mandated to be performed separately from the bulk form, and REACH guidance documents are being updated for NM. While ECHA’s efforts and other activities may eventually lead to effective guidelines to control NM risks, there is a need to implement state-of-the-art risk control through the nano-enabled product lifecycle. There are two key challenges to be addressed in implementing risk control through the nano-enabled product lifecycle. The first concerns the application of appropriate Technological Alternatives and Risk Management Measures (TARMM) to address the risk posed by a specific nano-form in an exposure context. The second concerns implementation of risk control in a cost effective manner, as even explicitly recognized by regulations (e.g. REACH Authorisation’s Analysis of Alternatives and Socioeconomic Analysis) and policy prescriptions (e.g. European Commission’s Precautionary Principle). In this context, in the frame of the SUN project, a risk control methodology that addresses the issues highlighted above was developed and implemented in the SUN Decision Support (SUNDS) system as one of the modules, named Risk Control (RC), in its higher tier. The RC module supports the control of human health and ecological risks by assessing risk control strategies (so called TARMM) through nano-enabled product lifecycle. The RC methodology is currently being applied to two case studies: 1) nano-copper oxide based wood preserving biocidal paint (compared to a conventional acrylic paint), and 2) plastic automotive part (bumper) coloured with two kind of pigments: nano-sized organic pigment and nano-sized carbon black, highlighting the difficulty of identifying successful risk management options when conservative risk estimations are available.

WE198

Human Health Risk Assessment along the life-cycle of nano-enabled products

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The SUNDS (SUN Decision Support system) web application software has been developed in the SUN European project on Sustainable Nanotechnologies (www.sun-fp7.eu). The software aims at supporting decisions on assessment & management of nanomaterials and nano-enabled products in industry, regulatory bodies and insurance companies. Within SUNDS, the Human Health Risk Assessment module implements a quantitative HHRA methodology for the estimation of deterministic and probabilistic risks caused by nanomaterials along different life cycle stages (synthesis, formulation, use and end of life). In the SUNDS HHRA module, risk is evaluated by combining the outputs of the effects (hazard information) and exposure models. The outputs of these models, as well as the resulting risk, can be estimated deterministically or probabilistically, depending on data availability. Risk is estimated for a combination of aspects including a lifecycle stage, a specific target, a specific activity (where applicable) and a defined route of exposure. This combination has been called "lowest unit of assessment" (LUA). With the aim of providing integrated information on the estimated risks for specific life cycle stages or for the entire lifecycle of a specific nanomaterial, aggregations methodologies are required. The aggregation methodologies implemented in the HHRA module produce a single risk value for each lifecycle stage as well as for the entire lifecycle considering all lifecycle stages, targets, activities and routes of exposure (i.e. all assessed LUAs). Moreover, they may be additive (in the case of risks related to the same target) or non-additive (in the case of risks related to different targets for the same lifecycle stage). The HHRA methodology has been applied to different case studies including nano-copper oxide -based biocidal paint, plastic automotive part (bumper) coloured with two kind of pigments: nano-sized organic pigment and nano-sized carbon black, nano-Silver used in antibacterial polymer fibres in textiles, Titanium Dioxide (TiO_2) for self-cleaning coating for ceramic tiles. The focus of this contribution will be on the results of the application of the developed HHRA methodology to the case study of nano-copper oxide -based biocidal paint and plastic automotive part coloured with nano-sized organic pigment.

WE199

Supporting the environmental risk assessment of nanomaterials with quality-approved information - the DaNa Literature Criteria Checklist

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Nanotechnology is of increasing significance for many sectors of industry opening the market for numerous new applications, ranging from electronics to health care and environmental remediation techniques. Nanomaterials offer a great innovative potential and they are assumed to be beneficial to mankind and the environment by e.g. reducing material usage and energy consumption. However, the large variety of synthetic nanomaterials developed in the last decade together with all current and future new (nano)materials represents a major challenge for scientists and regulators in terms of measuring and assessing the potential hazard caused by the materials and the products over the whole life-cycle. Addressing the issues of material characterisation and assay harmonisation being the key challenges of any nanosafety assessment and safe-by-design approach, the DaNa project team (*Data and knowledge on nanomaterials*) developed and recently updated its' Literature Criteria Checklist providing the nanosafety community with a useful tool for quality evaluation and management of scientific publications. This checklist includes mandatory and desirable assessment criteria covering the topics physico-chemical characterisation, sample preparation and necessary (biological) testing parameters ensuring a thorough, comprehensive and fit-for-purpose assessment of the used nanomaterial in any given setting (products, humans, environment). With this approach, the international DaNa-expert team has been evaluating scientific publications on nanomaterials dealing with safety issues for humans and the environment. All literature approved by the criteria checklist is then cited in the DaNa Knowledge Base and published on the website www.nanoobjects.info. This web platform offers easy-to-understand, up-to-date and quality-approved information on 26 market-relevant nanomaterials concerning their effects on safety of humans and the environment, making an important contribution to science communication in the field of nanosafety. *References:* Kühnel, D. et al. (2016) *Environ. Sci. Pollut. Res.*doi:10.1007/s11356-016-6217-0**Nau, K. et al. (2016)** ***J. Mat. Educ.*** **<strong>38** (3-4), 93 – 108.**D. Kühnel et al. (2014)** ***Environ Sci Eur*** **<strong>26,** 21. DOI: 10.1007/s11356-016-6217-0 *Acknowledgement* - The funding for the DaNa2.0 project is provided by the German Federal Ministery for Education and Research (BMBF) under grant no. 003X0131. Additional support by Swiss Federal Authorities is acknowledged.

WE200

Are there significant acute ecotoxicological effects of nanoparticles?

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The paradigm of environmental risk assessment of chemicals states that risk is to be quantified on a comparison of environmental concentrations and no-effect concentrations (PNECs). For the specific case of engineered nanoparticles (NPs) there is *a priori* no ground not to accept the risk assessment paradigm. In common practice, PNECs are based on chronic toxicity data generated for biota of different trophic levels. \n Various proposals have been brought forward regarding mechanisms of interaction and subsequent toxicity of NPs. Nevertheless, actual mechanistic understanding remains limited, and there is also no simple correlation between toxic response and NP properties. Some information suggests that subcellular endpoints, especially oxidative stress, may be more sensitive for NPs than for conventional contaminants. This information and other examples indicate a trend of subtle long-term effects of biota exposed to low levels of NPs, whereas particle specific acute toxic effects appear to be absent. On forehand, a number of factors may limit observation of acute effects: • A key challenge in NP testing is that exposure often is not constant because of particle setting and transformations that typically occur. Consequently, the simple two-dimensional approach for deducing steady state mass concentrations that express the exposure dose, is problematic for use with NPs; • Lack of understanding of how cells sense crystals; • Lack of NP specific markers of toxicity to confirm the presence of particles and their subsequent adverse effects; • Actual lack of acute effects – insufficient effective exposure and severe time restraints to reach critical levels at NP-specific sites of toxicity within biota. In light of the factors assumed to limit the occurrence of actually occurring adverse effects of NPs, the claims of acutely induced NP effects will be discussed in this contribution. To this end, we aim to categorize the available literature with regard to a limited number of classes of NPs, assess the effective exposure, and assess whether the effects observed can indeed be classified as being particle-imposed or whether the effects are indirectly due to the addition of NPs to the exposure medium.

LCA for supporting policy and decision making (P)

WE201

Consumer Survey and Life Cycle Assessment (LCA) of Pharmaceutical Waste Disposal Practices in the UK

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Unused or expired medicines from the hospital and household waste can ultimately end up in landfills or be released to the wastewater system. There is, therefore the potential for active pharmaceutical ingredients (APIs), from a range of medicinal products, to be present in landfill leachate and sewage effluents. In this project, a household survey was performed to understand the typical waste generation patterns for medicines and the disposal routes. The results from the household survey showed; 206 respondents from Yorkshire (56.3%), and 43.7% from Berkshire, Oxfordshire, Greater London and other regions. From the survey as well, the results have been divided into two categories, over-the-counter (OTC) and prescription medicines; disposal options order for OTC medicines from 53.2% of disposed of percentage are, throw into rubbish (28.6%), take-back to pharmacies (16.1%), toilet disposal (1.9%), sink disposal (1.6%) and others such as disposal as per instructed on medicines packets (5%); meanwhile for the disposal options order from 21.2% of disposed of percentage, take-back to pharmacies (12.1%), throw into rubbish (5.4%), toilet disposal (0.3%), sink disposal (0.3%) others such as disposal as per instructed on medicines packet (3.1%). It shows that disposal into rubbish (34%) has been identified as the most common disposal methods by the respondents in the UK and these pharmaceuticals waste will ultimately end up in the landfills. Life cycle assessment (LCA) was then used to compare the environmental emissions from different pharmaceutical waste disposal options. The LCA work is ongoing but is exploring the following practices: disposal to the toilet or sink (wastewater treatment); the use of take-schemes (incinerations); disposal of into rubbish (landfills); and new in situ treatment approaches (e.g. PyroPure). Conclusions from the analysis will be presented.

WE202

Life cycle Assessment of freight transport in Belgium

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BRAIN-TRAINS is a project supported by the Belgian Federal Government that deals with the possible development of rail freight intermodality in Belgium, analysing the current situation of the intermodal transport from an interdisciplinary perspective. Life Cycle Assessment (LCA) methodology have been used to analyse the sustainability impact of rail freight intermodality for three divergent Belgian scenarios by 2030. These scenarios are directly linked to the third strategic goal of the European Commission's White Paper on transport (2011), which aims to shift the 30% of road freight over 300 km to other modes of transport more energy-efficient such as rail or waterborne transport by 2030. As a result, a best, worst and medium case scenarios have been developed, depending on whether the 30% shift has been successfully accomplished, the status quo has been maintained or the goal has not been completely reached by 2030, respectively. The LCA of the intermodal freight transport in Belgium including rail freight transport, inland waterways transport and road transport has been carried out. A detailed study of the rail freight transport have been conducted, collecting data directly from Infrabel (the Belgian railway infrastructure manager) and B-Logistics, which is the main rail freight operator in Belgium. The rail freight system is divided in three sub-systems: rail transport operation, rail infrastructure and rail equipment. The specific energy consumption of electric and diesel trains has been determined separately. In order to adjust as closely as possible the environmental impact related to the yearly electricity consumption, and since the electricity supply mix varies widely over the years, our LCA study uses the electricity supply mix in Belgium corresponding to the appropriate year. In the case of both inland waterways transport and road transport in Belgium, we are using as a model the Ecoinvent database. Information relative to the total annual freight moved by inland waterways transport in Belgium by barge type, fuel consumption in the vessel transport operation and waterways infrastructure characteristics for several years have been collected. Similarly, information relative to the total annual freight moved by road transport in Belgium by weight classification and heavy duty vehicle technology type, fuel consumption in the road transport operation and road infrastructure characteristics for several years have been collected.

WE203

Life Cycle Assessment of an integrated system combining microalgae and constructed wetlands for the treatment of domestic wastewaters

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The present research aimed to apply Life Cycle Assessment (LCA) to different systemâ_s configurations for the treatment of urban wastewaters, combining Microalgae (MA), Vertical Flow Constructed Wetlands (VFCWs) and Horizontal Flow Constructed Wetlands (HFCWs). The functional unit was defined in m^3 of final wastewaters treated for 20 years. The boundaries of the system were delimited from the entry of the raw wastewater into the UASB reactor until the disposal of the treated wastewaters in the receiving body. SimaPro® 8.04 software and the Impact 2002^+ database were used for the LCA assessment considering 15 different impact categories for the construction and operation stages. The system was analyzed by applying midpoint (kg_{eq}) and endpoint characterization (Human Health, Quality of Ecosystems, Climate Change and Resources). By the application of the LCA, it was possible to identify the main impacts related to the construction and operation of these systems. The results showed that the highest environmental pressure indexes of the construction phase were related to the use of sand and plastic materials in the scenario of bench scale of MA + VFCWs, and plastic materials in the scenario of the pilot scale UASB + VFCWs and HFCWs. When considering the operation phase for the two scenarios, the main impacts were associated with the electricity use due to the dependence on non-renewable resources. The most relevant impact categories of the process were: aquatic eutrophication, global warming, carcinogenic and non-carcinogenic agents, aquatic and terrestrial ecotoxicity, soil occupation and non-renewable resources. The materials include global warming, carcinogenic and non-carcinogenic agents, aquatic and terrestrial ecotoxicity, land use, non-renewable resources and mineral extraction. The adoption of wastewater treatment systems using biological processes or phytoremediation emerges as a promising alternative for a more sustainable sanitation, presenting itself as a tool for the recovery of water and nutrients with lower environmental impacts. In this sense, the LCA allowed the identification of the highest environmental impacts of the construction and operation phases, enabling a complete diagnosis of the system and consequently allowing the adoption of measures that might improve the performance and reduce the impacts through the substitution of materials and processes.

WE204

Combined use of UVV and UVC photochemical reactors for the treatment of the hospital laundry wastewaters: life cycle assessment as a support tool for selection of the system's configuration

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Hospital wastewaters represent an unquestionable pollution source of the aquatic environments. In this scenario, the wastewaters generated at hospital laundries require special attention due to features like high COD, BOD_5, microbial load, and toxicity as well as high concentrations of particulate matter, proteins, starch, fat, oils and grease, detergents, disinfectants, and pharmaceutical products,. Therefore, aiming to develop a system for the treatment of hospital laundry wastewaters with greater efficiency and less environmental impacts, this research investigated the combined use of photochemical emerged (UVV) and immersed (UVC) reactors, including evaluation of the reduction of the pollutant parameters of the hospital laundry associated with the application of Life Cycle Assessment (LCA). The characterization of studied wastewaters included analysis of the following parameters: COD, BOD_5, TKN, total P, pH, turbidity and conductivity. Acute ecotoxicity was evaluated using *Daphnia magna*. Ultraviolet-Visible (UV-Vis) spectroscopy was performed to determine the organic fraction and chromatography coupled to the mass spectrometer (GC-MS) was used for the qualitative characterization of priority pollutants. Characterization parameters showed the presence of drugs like Lidocaine and Dipirone and a high organic load with a poor biodegradability. Wastewaters presented an extreme acute toxicity against *D. magna*. The ozonation process (mainly generated by the UVV reactor) presented the best results concerning the removed kWh/COD ratio, and the UVC process showed the lowest environmental impacts for the Characterization and Normalization parameters of the LCA. Normalization revealed the highest environmental burdens associated with human toxicity, surface water, ecotoxicity and eutrophication. It was also found that the use of low voltage electrical energy was responsible for the most of the 14 evaluated environmental impacts and the wastewaters were mainly related to eutrophication. UVV/UVC/O_3 process showed the best results considering detoxification. Cleaner production measures should prioritize the reorganization of the configurations of the combined UVV/UVC reactors, applying a Pitot-Venturi for the gas-liquid transfer. This will involve the construction of a prototype in a single tubular reactor, centralizing the reactors.

WE205

Life cycle thinking for the Brussels' circular economy transition

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This poster presents an ongoing research project financed by the Brussels Capital Region (BCR) in which material flow analysis (MFA), input-output analysis, life cycle thinking and optimization are used to support decision making in the valorization pathways of waste streams of the BCR. The general aim of this project is to analyze the economic and environmental potential of the waste streams for a transition towards a circular economy. This means we will identify which combinations of available waste streams, recycling and treatment technologies, and modes of entrepreneurship are the most promising in terms of the transition towards a more circular economy in the BCR. In this context the project will start from an overview of all waste streams in the BCR. A transversal theme of this project is the question whether a region wide waste management should combine domestic and industrial waste streams, or not. Would the scale of the material flow through combining domestic and industrial waste give more degrees of freedom in the organization of collection, recycling and treatment, or not? Would merging these streams render certain waste management systems economically and ecologically viable in the region itself? A region wide MFA will be performed by using a multi-region input-output model (MRIO) extended with the physical waste flows of the regional waste register. Although these MFA and MRIO will cover all waste flows of the BCR, only a selection of these flows will be subject to further analysis. In these further analysis we use life cycle assessment (LCA) and life cycle costing (LCC) to assess the environmental and economic performance of different waste valorization technologies for the selected waste streams. These different technologies will be selected based on technical feasibility and desirability in the BCR context. The eco-efficiency will be evaluated for both the current as future anticipated size of the selected waste streams (based on MFA and metabolic analysis). Further, the results of these LCAs and LCCs will serve as input for a multi-objective optimization model for finding (near-)optimal solutions in terms of facility location, waste collection (allocation and routing) and treatment. With this combination of modeling and impact assessment tools, we aim to support decision making at the BCR by providing technical-economic-environmental optimal waste valorization pathways which can be part of the BCR transition to the circular economy.

WE206

The connection of life-cycle thinking with the European Commission's Circular Economy Strategy in the Central Eastern European region

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The XI. annual life-cycle assessment (LCA) conference of LCA Center Hungary Association has been held on 22-23 november 2016 in Budapest, the first time with a strong international outlook to the Central Eastern European region. The event this year has been organised around the topic of life-cycle thinking in relation with the Circular Economy concept.\n More than 60 participants attended with almost the half representing various CEE countries other than Hungary but mostly from Romania and Serbia. Participants also came with a diverse background from a sectorial viewpoint as they included representatives of industry, government and academia. The Circular Economy concept has been recognized as an important milestone towards a resource efficient economy as well as a great vision to spread life-cycle thinking. However, participants have agreed that the EC's Circular Economy Strategy adopted a narrower, end-of-pipe viewpoint regarding waste management and does not address earlier stages of products' life cycle properly. Members of industry raised concerns over the many different bureaucratic and sometimes contradicting European and national regulation that create a barrier for efficiently move towards adopting a Circular Economy approach. They also noted that material recovery is sometimes not viable for technological or thermodynamical reasons. Participants emphasized the development of LCA networks in the CEE region by further strenghtening ties with neighbouring countries and integrating into a wider European and global LCA community especially considering the commencing LCM2019 conference to be held in Poznan, Poland and co-organized by CASE LCA network.

WE207
Recent advances in Life Cycle Assessment for soil and sediment remediation
V. Cappuyns, KU Leuven / Centre for Economics and Corporate Sustanability
Since 1999, LCA has increasingly been used to quantitatively evaluate the secondary environmental impacts of soil and sediment remediation activities. Some reviews published between 2004 and 2011 showed the possibilities and limitations of the application of LCA in the context of soil and sediment remediation. Most of these studies were dedicated to soils and sediments contaminated with organic contaminants and focused on ex-situ remediation. Other concerns are the need for the definition of an impact category including human toxicity via groundwater, and the lack of attention paid to potentially degradable contaminants forming toxic metabolites The use of LCA in de context of soils and sediment remediation has also severly been criticized because environmental effects of the post-remediation stage of the site is generally disregarded. It should be clear that (environmental) LCA cannot be considered a holistic decision-making tool for site remediation (i.e. including social and economic aspects), but has a clear focus on environmental and health impacts. The case studies performed between 1999 and 2011 also showed that the result of LCA is highly dependent on the impact asessment method used and that the choice of impact categories heavily affects the outcome of a LCA. In the present paper, the use of LCA in the context of soil and sediment remediation, as found in case studies published from 2012 until 2016, was evaluated. The scope of the LCAs is in most cases the assessment of secondary environmental impacts of the remediation activities, but there is no generally accepted functional unit. Climate change (expressed in GWP) and fossil depletion are often reported to be the most important impact categories for a soil/sediment remediation project. In some cases, impact asessments methods from more than 20 years ago are still used, whereas updates are available. The inclusion of impact categories such as land use and soil quality should be further developed and implemented. The former focus on LCA of site remdiations dealing with organic contaminants and ex situ remediation shifted towards case studies dealing with the remediation of sites contaminated with heavy metals, often using phytoremediation or other *in situ* technologies. Working out some well documented case studies, with more uniform functional units, including a proper sensitivity analysis, could stimulate the use of LCA in real-life applications.

WE208
USING LCA FOR DECISION-MAKING ABOUT A SUSTAINABLE ENERGY PRODUCTION: A CASE STUDY FROM FOREST RESIDUES IN PORTUGAL
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The Earth's climate is changing as a result of anthropogenic activity since the start of the industrial revolution. Most greenhouse gases (GHG) emissions come from electricity production and transportation, which are important sectors to national security and economic growth, indicating that the transition to a low-carbon economy will make a difficult political trajectory. To assure that the reduction of GHG emissions does not interferes in the country's economic development, the production of energy from forest residues has emerged as an option. In line with the European policy, in Portugal several power plants fuelled with forest residues have been built in the last few years. However, studies are needed to evaluate the impacts related to this activity. Despite the advantages of energy production from forest biomass, the use of forest biomass is associated with substantial cost to the environment, which can affect the human well-being. Life cycle assessment (LCA) has become one of the most important environmental indicator for measure and assess the related impacts on sustainability and to provide support for organizational decision-making activities. The goal of this study is to evaluate the environmental impacts associated with the production of electricity from forest biomass residues in Portugal. LCA methodology is applied as a decision supporting tool to select the technology with the best environmental performance. The functional unit is the production of energy equivalent to one kWh by converting eucalypt residues delivered to the power plant. System boundaries include the stage of forest management, transport and energy conversion at the power plant. This study assesses some of the environmental impacts of producing energy from eucalypt residues in Portugal. Moreover, it identifies the operations with the largest environmental impact. This analysis is particularly important given the forest residue market in Portugal and the increasing demand of forest residues for bioenergy. The results obtained for each impact category show a relatively wide range of variation when different management scenarios and wood productivities are taken into account. Finally, the main environmental decisions and current policy projections in Portugal are assessed. The methodology adopted here can also be applied to predict what is the technology with the least environmental impact, being applied as a decision supporting tool.

WE209
Human well-being as single endpoint in life cycle sustainability assessment, facilitating policy support
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A sustainability assessment evaluates the impact of a studied system on areas sought to be protected over time (e.g. mankind, ecosystems…). These areas are called Areas of Protection (AoP). Life cycle sustainability assessment is a type of sustainability assessment that focuses on the impact of industrial production systems on AoP. There are three conceptual challenges for this field for better policy support: (1) selecting which aspects should primarily be sustained and hence on which the impact should be assessed, i.e. (re)defining of the AoP, (2) accounting for the interlinkages among AoP (e.g. influence of ecosystems on human well-being) and (3) the assessment of both benefits and damages on the AoP (e.g. damages related to industrial production and benefits associated with their industrial products). This study outlines a first roadmap to address those three issues. Concerning the first challenge, our hypothesis is that human well-being, encompassing health and happiness, shall be considered the only final AoP. This is based on the argumentation that the sustainability concept is inherently anthropocentric. In light of human well-being, other entities such as ecosystems are sustained. Regional or individual differences in the preference on what shall be sustained, and the associated practices, are possible but only if they do not jeopardize the global human well-being. It is also necessary to provide a single outcome for decision support. The Well-being adjusted life years (WELBY), introduced by health scientists and interpreted as years of perfect well-being, is therefore pinpointed as the most promising holistic and tangible single indicator to assess overall sustainability. Not only should the total global WELBY be considered but also that of individuals since there is a need to strive for an equal distribution of well-being. However, well-being should be discounted over time, since current generation well-being is potentially neglected compared to well-being of endless future generations. To conduct a respective sustainability assessment that tackles also the other two issues, integrated Earth assessment modelling is proposed as a suitable approach. While this study outlines new conceptual steps in the field of life cycle sustainability assessment, there is a clear need for further research and consensus by a broader community.

WE210
Sustainable recycling in the industrial production of foam glass
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The growing awareness regarding recycling issues, led to the development of innovative techniques to reintegrate several times the waste materials in the production cycle. Turning waste into a resource is one key to a circular economy. European legislation improve waste management, stimulate innovation in recycling, limit the use of landfilling, and create incentives to change consumer behavior. In Europe, each year, approximately 1.5 million tons of glass waste is generated by the demolition and renovation of buildings, and almost 11.5 million of tons of glass packaging was collected all over Europe. The glass recycling operations have proved to be very advantageous both from an economic and environmental point of view. However, it must be considered that in preparing suitably clean cullets for recycling in the glass process, 23 to 25% of such amounts currently end up as waste in landfills. Even if waste differentiation and collection is increasingly becoming a good practice in Europe, there is still a lack of proper recycling technologies from a feasibility, environmental and economical point of view. The aim of this study is to assess the environmental performance of expanded

building materials, namely foam glass, with outstanding insulating properties, using up to 95% waste materials deriving from silica-based waste like metallurgical fly ashes, undifferentiated glass, glass ceramics materials and small quantities of porcelain. An innovative spacer method has been also used by admixing NaCl to the atomized powders in order to create a percolating dispersion of such salts, which remain almost unaltered during the reactive oversintering of the surrounding material. Such salts are then removed by solubilisation in water, thus leaving in the material pores of regular shape and an open porosity not achievable by any other method. The LCA analysis was conducted from a cradle to the gate perspective using the SimaPro 8.0.4 software and the IMPACT 2002+ evaluation method. The functional unit is 1kg of the mixture producing a foamed panel of 0.16m^2 and 0.0125m of thickness. The inventory analysis has been conducted using primary data whenever available. The remaining data have been obtained from the EcoInvent database. The results show as the foam product obtained by waste glass has an environmental burden of 54.14 mPt and it reduces the environmental impact of 49% with respect to the traditional foam glass.

WE211
Embedding Sustainable Strategy into Innovation: L'Oréal's sustainable product assessment tool
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Launched in 2013, « *Sharing Beauty With All* » program defines L'Oréal's 2020 vision and commitments in terms of sustainability. L'Oréal has targets for four key areas: Innovating sustainably, Producing sustainably, Living sustainably and Developing sustainably. Regarding sustainability performance at product level, L'Oréal is notably committed to: develop and launch a product only if its environmental or social performance is improved; provide data to consumer on the environmental and social performance of its products. These commitments rely on a product assessment tool designed for all cosmetic products of the Group. This tool allows the assessment of an overall sustainability profile per product, based on its quantitative environmental footprint as well as on its social impact. The assessment of products relies on a life cycle assessment methodology, which covers all life stages of each product. The tool integrates life cycle management (LCM) at the core of L'Oréal's global strategy. In order to ensure robustness and recognition, the methodology has been established with an external stakeholder panel and in agreement with international standards such as ISO 14040 and 14044, European Commission ILCD handbook and Commission Recommendation of 9 April 2013 on the use of common methods to measure and communicate the life cycle environmental performance of products. The first approach of the tool was presented at SETAC LCA 2016. The methodology has evolved according to new data, new developments and results. During the presentation, several strategic and methodological aspects will be addressed, including: The use of multicriteria assessment (environmental indicators in line with the Product Environmental Footprint (PEF) guidelines) The aggregation of all environmental impacts into an aggregated environmental impact using the "Planetary Boundaries" concept as a weighting framework The development of databases in order to allow the coverage of all L'Oréal products, packaging and Formula ingredients, requiring significant development in order to expand the coverage of existing databases. The adaptation of USETox methodology to cosmetic products. The sustainable product assessment tool is a powerful tool that embeds sustainability metrics into day-to-day innovation: Product designers will be able to assess if a new design complies with L'Oréal sustainability strategy.

WE212
Efficiently performing a consequential Life Cycle Assessment on site remediation alternatives
L. Huysegoms, V. Cappuyns, KU Leuven / Centre for Economics and Corporate Sustanability
Site contamination is a global concern because of the potential risks for human health and ecosystem quality. In Europe alone there are 340 000 potentially contaminated sites and this number is forecasted to increase even more by 2025. The huge amount of contaminated sites that will have to be remediated in the coming years has increased the attention for the secondary environmental impacts (i.e. the environmental impacts caused by the site remediation activities themselves) of the remediation. These secondary environmental impacts are also an aspect of the more holistic 'sustainable remediation' framework. Every contaminated site has its own specific characteristics and the large range of potential contaminants, as well as the recent focus on sustainable remediation, resulted in new developments, and stimulated technological innovations. The increased availability and efficiency of techniques makes the final choice of remediation alternative increasingly complicated. Life Cycle Assessment (LCA) can support site owners and remediation companies in the choice between remediation alternatives, taking into account their secondary environmental impacts. LCA as such has also been used on several case studies to compare the secondary environmental impact of different alternatives but has been critiqued for being too complicated, time consuming, and data demanding. This paper shows how, by following the ISO 14 040 and 14 044 guidelines initially drafted for the development of a consequential LCA, a consequential study of the environmental impacts of a site remediation case study can be done more efficiently even with little previous experience in performing LCAs. The case study used for this purpose entails the soil and groundwater remediation of a tar, poly-aromatic hydrocarbons (PAH) and cyanide contamination of a school ground by a former gas plant. Two remediation alternatives, described in the preliminary soil investigation report, namely, two excavations with each a different volume of soil to be treated, were evaluated with LCA. The consequential LCIA is based on the data from the site remediation project and modeled by using the Ecoinvent 3 database and ReCiPe method within SimaPro 8.2.0.0. Based on this analysis, the preferred remediation alternative is excavation with the smallest volume of contaminated soil with off-site thermal treatment, mainly because this alternative requires less transportation of contaminated and clean soil from and to the site.

WE213
Life Cycle Assessment approach in prefabricated-wood-frame buildings
A. Padilla-Rivera, Universite Laval; P. Blanchet,
In the las decade growing population has demanded the design and construction of buildings, which requires integral and environmentally efficient building systems. In this sense the Intergovernmental Panel on Climate Change (IPCC) reported that buildings accounted for 32% of total global energy use, 19% of total GHG emissions, approximately one-third part of black carbon emissions, and an eight to a third of F-gasses. Furthermore, the assessment of impacts of the building activities as well as minimizing building costs, selecting materials and reducing wastes are becoming nowadays some additional goals in order to mitigate GHG emissions. In this paper, a quantitative life cycle model for green house gas emissions accounting was developed based on the life cycle assessment (LCA) theory. A residential wood-frame building in Quebec City in Canada was selected as a baseline scenario, it has been constructed according to the concept of green environmental protection and sustainability practices. In the baseline scenario of this research, material production and assembly construction phases were assessed by TRACI 2.1 impact assessment method. Additionally, six alternative scenarios were proposed in order to evaluated different construction building parameters. The most important parameters are considered in a materials flows study about their energy, water, materials and waste. The results of this study confirm the positive effect using prefabricated approach in buildings as an alternative construction method based on wood-frame-materials in Quebec. They relatively reduced environmental burdens were remarkable for all stages. According to the environmental evaluation presented, baseline scenario produces (275 kg/ m^2 CO_2 eq.) 25% less than traditional buildings. Meanwhile, materials production phase is the most significant contributor to the life cycle approach (75%), being the insulation and sealing materials (28%) and concrete (17%), used for the construction of the building's foundation, covering and basement, the principal generators of GHG emissions. The findings indicate that prefabricated wood-frame building is an alternative environmentally friendly option, above concrete or steel frame options, however, it is fundamental to promote a radical change in waste management practices of the building, this involves committing decisively to reuse and recycling of different materials, and always minimising the transport of the starting materials and products, promoting the use of resources available in local areas.

WE214
Life Cycle in Practice - Helping SMEs to integrate life cycle approach into their policy
S. Groslambert, University of Liège - Chemical Engineering / Dpt of Chemical Engineering - PEPs; A. Leonard, University of Liege
The application of Life Cycle Approaches – including Life Cycle Assessment, eco-design and environmental labelling – is becoming an increasing reality for business, and a growing challenge in many economic sectors. Businesses are facing increasing legal and market requirements to enhance resource efficiency and reduce the environmental impact of their products & services. To significantly address this challenge, the Life Cycle in Practice project (LCiP - LIFE12 ENV/FR/001113) was conceived, aiming to promote the uptake of LC approaches particularly in SMEs. The overall aim of the LCiP project is to help SMEs in France, Belgium, Portugal and Spain in reducing the environmental impacts of their products and services across the entire Life Cycle and to foster the implementation of circular economy in these regions. Three sectors are selected: Buildings & Construction, Waste Management and Energy Equipment. Thirty-two businesses are involved in the four regions, twelve being located in Wallonia. GreenWin, the Walloon partner, has mandated several teams well-known for their expertise in LCA to coach them. ULg-PEPs coached three SMS's to help them to carry out the Life Cycle Assessment (LCA) of their product and accordingly to identify the strengths and weakness of the products and/or processes. Two of them are involved in building insulation (hemp con-crete blocs from IsoHemp and sound insulation panels Acoustix made by Pan-Terre), and the third one is developing an accelerated composting equipment for food waste (EcoCleaner). Concretely, the LCA of the three products are realised in a cradle to gate perspective. The results show possible environmental impact improvement for all the products, even if they are eco-designed. As a coach, the experience of working closely with highly motivated people in small structures is very gratifying and a smart way to help life cycle approaches to develop in our region. We are pleased to go on this initiative by

hosting one of the Physical Resource Centres in the University of Liège to welcome SMEs seeking to integrate LC approaches into their businesses, and give them access to selected LCA tools, reading material, expertise, training and advice. Results and Online Resource Centre are centralised on the LCiP website: http://www.lifelcip.eu/.

The challenges of Life Cycle Sustainability Assessment (LCSA) of energy technologies (P)

WE215

Sustainability issues of a new building integrated PV system

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REELCOOP, an EU-FP7 funded project which stands for REnewable ELectricity COOPeration (www.reelcoop.com), aims to develop renewable electricity generation technologies and promote cooperation between several EU Partners. It addresses five important renewable energy areas: photovoltaics (PV), concentrated solar power (CSP), solar thermal (ST), bioenergy and grid integration, with developing and testing three novel prototype systems are representative of both micro-scale (distributed) and large-scale (centralised) approaches to electricity generation. One of the prototypes is a solar PV ventilated façade (6 kW electrical output) and involves the development of both c-Si and dye-sensitized solar cells, as well as the study of the ventilation effect in PV façades. This prototype is currently installed at Yazar University (Izmir, Turkey - 2016). This work presents several indicators regarding energy costs and CO2 emissions savings, after carrying out an exploratory Life Cycle Sustainability Assessment. Preliminary results are compared to existing building integrated PV systems.

WE216

Exploratory analysis of the potential effects of criticality on solar and wind energy technologies in Spain

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Concepts of 'critical raw materials', 'criticality', 'resource criticality' or 'critical rare earths', and so on, have arisen as both research and policy issues due to the future importance not only in economic but also in social and environmental world development. These concepts are related to those raw materials which are economically and strategically important for the world economy, have a high-risk associated with their supply, and there is a lack of viable substitutes. Energy systems, like PV solar panels or wind turbines, have particular metals or rare earths that are potentially included in these raw materials. This work aims to: a) bring to light the most updated investigation and conclusions carried out by research groups and associations (European Commission or Critical Raw Material Alliance), b) list the potential improvements to develop a more comprehensive methodology, including new aspects like quantification of both risks or environmental effects, and c) state the potential mid-term effects on solar and wind energy technologies in Spain.

WE217

Sustainability assessment of biofuels production in Uruguay

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A sustainability life cycle assessment of various biofuels production chains in Uruguay has been carried out. The objective of this work was to perform a Life Cycle Assessment of the biofuels production chain in Uruguay with a special focus on GHG emissions and fossil energy consumption. The assessed pathways include, biodiesel production from oil seeds and fats and used oils as well as bioethanol production from sugar cane and cereals. Additionally an estimation of the socioeconomic impacts associated in terms of job creation, value added creation and impacts on the overall economy has also been performed. In this project, preliminary estimations of the externalities associated to the environmental and socioeconomic benefits have been performed. The analysis is based on actual data of biofuels plants in Uruguay.

WE218

SETAC Sustainability Interest Group

H. Breunig, Lawrence Berkeley National Laboratory

The Role of Metals in Circular Economies: A Life Cycle Perspective (P)

WE219

Granularity in Structural Steel Life Cycle Assessments

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As a material that can be recycled over and over again without significant loss of quality, steel is a key material for the circular economy. In construction applications, steel is desirable due to its design flexibility, high strength and durability, and aesthetic appeal. Scrap steel from structural applications is almost totally recycled and fit for repeated recycling. Consequently, it might seem that the contribution of steel to circular thinking is clear-cut and steel does not require specific attention through Life Cycle Assessment (LCA) studies. Contrary, our experience in construction steel LCAs for Environmental Product Declaration (EPD) development has shown that the amount of detail exercised can significantly affect conclusions and learnings from these studies. Steel construction products do not consist of one material only. Alloys, although small in quantity, are a key input into the manufacturing of steel construction products. Historically, alloys have not received much attention in steel LCAs and EPDs and certain alloys such as silicomanagenese have been omitted. This approach leads to an underestimated environmental impact of the products under study and overlooks opportunities in supply chain optimization. Furthermore, we find that the impact of steel products ties very closely together with mill-specific circumstances, since it is driven largely by the local grid mix. Investigating mills individually, in contrast to industry average or even manufacturer average studies, can provide valuable insights regarding plant efficiency and local conditions. We advocate for a granular and detailed approach to steel LCAs as it facilitates the identification of hotspots and reduction opportunities hereby supporting the construction industry in meeting the sustainable development agenda.

WE220

Comparative Life Cycle Assessment of the Production of SmCo and NdFeB Rare Earth Permanent Magnets

G. Bailey, KU Leuven / Material Sciences; B. Sprecher, Yale University; K. Van Acker, W. Dewulf, KU Leuven

Over 40 years ago, samarium was the rare earth element (REE) which dominated the rare earth magnet market, but now it is neodymium which is most often used in high performance permanent magnets. Samarium-cobalt (SmCo) permanent magnets tend to be costlier than neodymium-iron- boron (NdFeB) magnets. But more critical REEs must be added to neodymium alloys in order to be used at high operating temperatures safely, which could add to the increasing strain on these rare metals. The perception is that samarium cobalt is more expensive than neodymium iron boron due to shortage of supplies of cobalt in the past, but a few years ago, during the REE crisis of 2010, it was SmCo which was cheaper. Nowadays, magnet making industry find themselves employing the "less" critical metal to mitigate potential supply risk. In addition to criticality, the overall environmental impact however also needs to be considered. In this paper, using life cycle assessment (LCA), we compare the environmental impacts of the virgin production route for both neodymium iron boron and samarium cobalt magnets Our life cycle assessment is based on the LCA completed in 2014 [1] and on recent literature and industry data. While there has been a plethora of life cycle assessments performed for NdFeB magnets, SmCo magnets have generally been ignored. We also analyze the toxicity rating of these metals

WE221

How to integrate resources in LCA based on the real life of metals? Case of photovoltaics panels

S. Belboom, University of Liège - Chemical Engineering / Chemical Engineering PEPs; D. Michel Prieto, University of Liege / Chemical Engineering; A. Leonard, University of Liege; E. Pirard, University of Liege / Department Argenco

Resources, and more specifically mineral and metal resources, are on debate concerning how to model them in a LCA approach. Impact assessment methodologies are already taking into account the metals consumptions but using a modelled pathway which does not reflect the real life of metals. It usually assumes that the consumption of metals implies the decrease of the available amount of these metals and then the scarcity of the resources which is not fully the case, thanks to recycling. Furthermore, some metals are missing from the characterization factors (Gallium, Selenium, Indium, etc.) and then do not impact the environmental score of products. This study aims to highlight the needed work to reflect the right way to take into account metals based on a case study and proposing some ways to investigate in a next future. This study compares, using LCA, the environmental impacts of photovoltaics from both different technologies: multi-Si panel and CIGS panel. Both technologies are fully different in terms of involved metals and are then good examples to illustrate the approach. First step is to apply the available knowledge concerning the resources impact assessment methods to a full and complete inventory of both technologies and to highlight differences or similarities between methods. It also highlights the missing metals or minerals and then the effect on the global impact. The second step is the understanding of how metal resources are modelled. Based on this knowledge, using geological considerations and markets reality, ways of improvements are given for the modelling step. The final step is to develop new impact factors for missing metals and then to be able to have a fair comparison between technologies which were advantaged by the missing impact factors. This study is then a good example of an interdisciplinary work which gathered chemical and geological engineering knowledge but also economic considerations which are integrated in the resources and reserves definition used in the modelling of resources impact pathway. \n \n

WE222

Comparison of methods for dealing with multi-functionality in a recycling context

E. Bracquene, W. Dewulf, J. Duflou, KU Leuven / Department of Mechanical Engineering
Integrating recycling into an LCA is not straightforward but increasingly important to understand the benefits and burdens associated with recycling activities. One frequently discussed challenge is dealing with multi-functionality for co-products or processes with multiple in- or outflows. In practice, partitioning is often applied for the open loop systems even though it should be avoided according to ISO. The methodological choice between different approaches for dealing with multi-functionality in a recycling context may significantly impact the final results and influence the ranking of alternatives. Based on a **literature study**, most commonly aplied methods for dealing with recycling are identified.The review will also focus on the **different interpretations** that practitioners give to same named methods. For instance, 'system expension' is assimilated by some practitioners to 'substitution' or 'credit awarding'. However other practitioners make a clear distinction between these two actions : including the multiple functionalities to the functional unit and substracting avoided burdens. The study will **highlight advantages and trade-offs** related to chosen methods. Although the cut off method is straightfoward and easy to use because there is no need for additional data outside the observed product system, it does not reflect the full consequences of recycling as it ignores the relationships between virgin and recycled products and the fact that the recycled products would not exist if the virgin product was not manufactured first. In contrast, the system expansion needed to avoid partitioning requires a substantial amout of data across the different, not always well-known, product lifecycles but it results in a wholistic approach insuring there is no burden shifting. On the downside, it provides an aggregated result with limited added value for further detailed interpretation and analysis. Additionally some **comon practices**, such as the use of avoided burden method in attributional LCA, are **critically reviewed and evaluated** and some key issues, such as identifying appropriate allocation factors, are discussed. The described allocation methods are applied to a **concrete recycling example** and a sensitivity analysis is performed to evaluate the impact of different model parameters. A LCA study on the end of life treatment of photovoltaic panels is conducted with focus on recovering and recycling of **valuable metals** (silver and copper).

WE223

Assessing selected metals flows in France and their recycling potential

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In a context of increasing global demand of metals (e.g., for building and high technologies), European countries, especially France, widely depend on foreign imports. This situation implies a real risk of physical or economic disruption of supplies. The European commission registered critical materials in a list for which data concerning flows should be improved and reliable. However, it is complex to calculate material balance of a substance at a geographical scale (including import, export, waste, stocks, recycling). In this study selected metals flows in France are assessed with the aim of evaluating the potential of increasing secondary raw material sources. The focus of the study is initially on bulk metals such as copper and iron and at a later stage at minor metals such as rare earth elements. \n \nCurrently, two main methods are used to map the flows of metals: Input-output tables (IOT) which is a top/down approach and Material Flow Analysis (MFA), which is a bottom/up approach. The IOT represent the exchanges in monetary value allocated to different economic sectors. These values are contained in a square matrix showing the monetary exchanges of materials between the activities. After conversion from price flows to physical flows, we obtain physical input-output tables (PIOT). Nevertheless, there are limits on the details how stocks and waste flows can be calculated with these tables. On the other hand, the aim of MFA is to quantify the whole flows (inputs, outputs, stocks) of a substance (or a product) at a process scale with defined boundaries. However, we do not necessarily know the flows for each process, and missing values would generate inconsistencies. A reliable "mapping" of metal flows from production to final use could be obtained by coupling these two methods. \n \nWe want to obtain meso-scale data to build a MFA for each metal. The goal is to reduce the uncertainties regarding material balance in order to make it clearer and more transparent while keeping the advantages of the systemic IOT and the precision of the MFA in an innovative approach. Once the new MFA completed, the results obtained are compared to the results from the SUIOT disaggregation. We provide a first attempt for obtaining data stemming from input-output tables and processes linked together by MFA in order to have an innovative map selected metals flow in France. Based on these data, foresee to evaluate the potential of increasing secondary raw material sources.

WE224

Integrated economic and environmental assessment as driver for innovation of metallurgical systems for the recovery of metals from low grade input materials

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Low-grade primary ores and secondary industrial residues often yield significant amounts of (critical) metals. As Europe doesn't have easily accessible deposits of raw materials, these low-grade resources could serve as a reliable source of metals. Traditional pyro- and hydrometallurgical methods do not suffice to exploit metals from these resources. Within the METRGROW+ project the main aim is to valorise the low-grade resources by implementing novel metalurgical systems, with a zero waste approach in which valorisation of all outputs of the raw materials is achieved. In order to reach that aim, a decision support tool including a iterative multi-criteria analysis that integrates Life Cycle, Risk and Techno-Economical Assessment (LCA-RA-TEA) to support technology development. Importantly for the LCA-RA-TEA is to align the goal and scope of the assessments. The goal of the integrated LCA-RA-TEA is to quantify the environmental impacts, environmental risks and economic impacts of the metallurgical systems that consist of the novel unit operations that are developed during the course of the project and tested for four different material streams (laterites, fayalitic slags, iron rich sludges and fine grained poly-metallic sludges). Impacts from the complete metallurgical systems will be assessed by combining results for the different unit processes. Many unit processes are still in an experimental phase, and impacts will be assessed based on outcomes of the different unit processes modelled to industrial scale. For the LCA-RA-TEA study a gate-to-gate approach will be used. The system boundaries of the studied metallurgical systems comprise the pre-treatment and the smelting-refining steps of the raw materials to the production of refined metals and matrix products. Downstream uses of the matrix products will be included in the system boundaries, as these will comprise novel products/uses/risks and could be different for the metallurgical systems investigated. The functional unit will be defined as the treatment of 1 tonne raw input material for the recovery of valuable metals (e.g. Cr, Ni, Zn & Co) and matrix products. This also highlights the zero waste concept that is key to the METGROW+ project. The product environmental footprint guidelines will be used as base for the LCA impact analysis. The outcome of the multicriterea LCA-RA-TEA will allow to select the most cost-effective and environmentally-friendly treatment for a given low-grade resource.

WE225

Environmental assessment of rare earth elements recycling: The case of recycling of end-of-life fluorescent lamps

D. Kontoulis, KU Leuven / Department of Materials Engineering; D. Dupont, KU Leuven / DEPARTMENT OF CHEMISTRY FACULTY OF SCIENCE; K. Van Acker, KU Leuven
Europium (Eu) and Yttrium (Y) represent some of the most critical rare earth elements concerning current supply and availability. Both elements are mostly used in phosphor powders which are contained in fluorescent lamps. The supply risk of the above rare earths, has put in the forefront the development of new green and efficient recycling technologies for end-of-life products like fluorescent lamps. At the same time, waste elimination and avoiding the burden of mining, with the concerns about radioactive elements like Thorium and Uranium which are present in ores and for which strict regulations apply, is strived at. This study has the aim to assess the environmental impacts, when end-of-life fluorescent lamps are recycled with different newly developed recycling technologies. The final purpose is to enable decision makers at company and governmental level, which of the proposed recycling routes to adopt and further implement. The modelling was performed using Simapro software version 8.0.3.14 and the life cycle inventory was based on existing literature and on own experimental data. Two different recycling processes were analyzed for the recycling of phosphor powder from end-of-life fluorescent lamps. The first one is a green and efficient new recycling technology, consuming only a limited amount of chemicals and electricity with no waste elimination, while the second one includes more process steps. Our LCA shows less environmental burdens for the first process, while impacts from waste are at a minimum for both recycling routes. Finally, despite the generation of waste in the second recycling route, most analyzed impact categories were dominated by burdens resulting from the use of chemicals and energy and not from the generated waste.

Regulatory Best Practices for Assessment of Endocrine Active Substances (P)

WE226

Applying Adverse Outcome Pathways (AOPs) to Derivation aquatic PNECs for Two Typical Phenolic EDCs

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More recently, adverse outcome pathways (AOPs) has been proposed as a novel concept that effectively take the consideration of toxic mode of action (MoA) and aid to guide ecological risk assessment (ERA) of chemicals. Assessing ecological risk of endocrine disrupting compounds (EDCs) is increasingly important due to its ubiquity in aquatic environment and endocrine effects on organisms. In the present study, from the point view of AOP, we derived aquatic predicted no-effect-concentrations (PNECs) of two typical phenolic EDCs, i.e. bisphenol A (BPA) and 4-nonylphenol (NP). Laboratory chronic toxicity data for BPA and NP on aquatic organisms were collected and grouped by whether they are associated with AOP, based on which endpoints include not only reproductive effects but also biochemical, genetic effects. By log-normal statistical extrapolation method, we calculated aquatic PNECs of BPA with values of 0.6 and 7.04 μg/L, and NP with values of 0.22 and 3.9 μg/L for AOP-related and non-AOP-related endpoints,

respectively. The present results showed that classification of toxicity data can provide lower AOP-related PNECs and keep aquatic organisms from adverse effect such as endocrine disrupting effect more appropriately. The ecological risk assessment framework raised in this study can also be applicable for other types of EDCs with different molecular initiating events such as binding with androgen receptor and thyroid hormone receptor.

WE227
Testing and assessment of endocrine disrupting effects of chemicals under Japanese programs: from EXTEND2010 to EXTEND2016
K. Yamazaki, Ministry of the Environment / Environmental Health
Ministry of the Environment, Japan (MOEJ) has been conducting testing and assessment of endocrine disrupting effects of chemicals under the program "EXTEND2010" since July 2010. In this program, the framework for testing and assessment of endocrine disrupting effects of chemical substances was established and test methods were developed under international cooperation, contributing to the establishment of international standard test protocols using fish, amphibians, and invertebrates. Under this framework, existing literature was evaluated and relevant *in vitro* and *in vivo* tests were conducted, which resulted in accumulation of knowledge of over one hundred chemical substances. MOEJ published its new program as "Further Actions to Endocrine Disrupting Effects of Chemical Substances – EXTEND2016 –." Following the basic policy already adopted in EXTEND2010 "Aiming to conduct environmental risk assessment properly and then to implement appropriate risk management measures on endocrine disrupting effects of chemicals substances, establishment of assessment procedures and implementation of assessment should be accelerated," relevant activities should steadily be implemented and then appropriate measures should be taken. Outline of the EXTEND2016 and updated state of the studies including significant results on testing and assessment will be presented at the Annual Meeting.

WE228
SYRINA - A proposed framework for the systematic review and integrated assessment of endocrine disrupting chemicals
M. Agerstrand, Stockholm University / Environmental Science and Analytical Chemistry
Endocrine disrupting chemicals (EDCs) have received wide attention from both the scientific and regulatory communities. Transparent and objective approaches to draw conclusions about the strength of evidence linking EDC exposures to adverse health or environmental outcomes are needed. Systematic review methodologies are ideal for addressing this issue as they provide transparent and consistent approaches to study selection and evaluation. The SYRINA framework, short for systematic review and integrated assessment, was designed for use with the International Program on Chemical Safety (IPCS) and World Health Organization (WHO) definition of an EDC, which requires appraisal of evidence regarding 1) association between exposure and an adverse effect, 2) association between exposure and endocrine disrupting activity, and 3) a plausible link between the adverse effect and the endocrine disrupting activity. The SYRINA framework includes seven steps: 1) Formulate the problem; 2) Develop the review protocol; 3) Identify relevant evidence; 4) Evaluate evidence from individual studies; 5) Summarize and evaluate each stream of evidence (i.e. epidemiology, wildlife, laboratory animal, in vitro, and in silico data); 6) Integrate evidence across all streams; 7) Draw conclusions, make recommendations, and evaluate uncertainties.

WE229
Assessing the Potential Impact of an Amine Oxide Surfactant on Estrogenic, Androgenic and Aromatase Endpoints in a Fish Endocrine Screening Assay
G. von Mérey, Monsanto Europe S.A. / Regulatory; S.L. Levine, Monsanto Company / Global Regulatory Sciences
Amine oxides (AO) surfactants have a diverse set of applications with 95% of the volume produced in North America used in household cleaning products (e.g., dishwashing, laundry detergents and hard surface cleaners). Because of their wide-spread use they are classified as a high production volume class of compounds in several member countries by the Organizations for Economic Co-operation and Development (OECD). AO surfactants have a low potential to bioaccumulate in aquatic tissues and the AO that was tested demonstrated relatively low aquatic toxicity. These characteristics minimize the potential for environmental exposure and toxicity to aquatic organisms. The purpose of this investigation was to evaluate the potential for estrogenic, anti-estrogenic, androgenic and impacts to steroidogenesis from an AO surfactant using a model aquatic vertebrate. Based on the non-cyclic structure of this AO surfactant, it was not predicted to be a ligand for the estrogen or androgen receptors or to disrupt steroidogenesis at environmentally relevant exposure concentrations and this is consistent with mammalian studies which have shown no reproductive and developmental effects. Fathead minnows were tested following the OECD 230 test guideline at concentrations of 0.14, 1.4 and 14 mg/L in a flow-through system after a 21-day continuous exposure. It was established in 14-day range-finding study that the highest test concentration did not result in overt toxicity. Endpoints used to assess activity were secondary sexual characteristics (tubercle scores) and vitellogenin levels (VTG). There were no effects on survival, growth, secondary sexual characteristics or VTG levels. Based on the endpoints evaluated, this AO surfactant has been shown to not have estrogenic, anti-estrogenic, androgenic effects or produce aromatase inhibition in fish, which confirms initial predictions based on its structures and results from mammalian studies for structurally similar AO surfactants. The no observed effect concentration (NOEC) was concluded to be 14 mg/L, a level that greatly exceeds predicted aquatic exposure concentrations.

WE230
Tier 1 Endocrine Disruptor Screening Program (EDSP) Assays and Regulatory Safety Studies Provide a Weight of Evidence that Glyphosate is not an Endocrine Disruptor
S.L. Levine, Monsanto Company / Global Regulatory Sciences
The EDSP includes 11 validated Tier 1 *in vitro* and *in vivo* screening assays that evaluate the potential for a chemical to interact with endocrine pathways. The endocrine screening system was designed to have redundancy between the mammalian and aquatic systems. Glyphosate was in the initial group of compounds that received test orders for screening under the EDSP. The compounds in this initial group were selected based on exposure potential and not on known or suspected interaction with the endocrine system. Results from the *in vitro* assays determined that glyphosate is not (anti)-estrogenic or (anti)-androgenic and does not impact steroidogenesis. These findings are fully consistent with peer-reviewed studies using functionally equivalent *in vitro* assays and predictions from structure activity relationships. Additionally, the *in vitro* results from the Tier 1 assays are consistent with the *in vivo* results from existing regulatory safety studies that provide information of the same nature. These higher Tier regulatory studies can provide definitive evidence of endocrine disruption or lack thereof, and are the most powerful tool for hazard identification. Consistent with the results from several multigeneration rat studies, glyphosate exposure had no impact on estrogenic, androgenic, steroidogenic and thyrogenic endpoints in *in vivo* mammalian assays, including the Uterotrophic, Hershberger, and male and female pubertal assays. Consistent the pubertal assays, the amphibian metamorphosis assay found no impact on thyroid-driven developmental endpoints. Results from the short-term fish reproduction study were consistent with the results of an existing fish full life cycle and showed no impact on all measured endpoints. From the weight of evidence provided by the Tier 1 assays, performed at independent labs, under the EDSP along with the higher Tier regulatory safety studies, it can be concluded with a high level of confidence that glyphosate is not an endocrine disruptor.

WE231
The Importance of Weight of Evidence Considerations for Assessment of Potential Endocrine Activity: A Case Study for Carbaryl
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Carbaryl was included in the first list of chemicals to undergo Tier 1 screening in US EPA's EDSP. Fish short-term reproduction assay (FSTRA) secondary male sex characteristics and gonadal histopathology data were interpreted as being suggestive of potential interaction with the androgen pathway in male fish. An EDSP Tier 2 Medaka Extended One-Generation Reproduction Test was suggested to be potentially useful based on these findings. The purpose of our analysis was to determine if potential endocrine responses in fish as seen in the FSTRA might be secondary to ChE inhibition. An extensive review of the extant literature, standard guideline study reports, and relevant US EPA regulatory documents was conducted. In male fish, there was an increased incidence of testicular degeneration as percentage of spermatogonia in the highest nominal treatment. There were no histopathological findings in females. There was a slight decrease in the median tubercle score (34 vs. 29 for controls vs. high dose, respectively). However, the remainder of the measurements related to endocrine modulation were negative. In addition, a fish full life cycle (FFLC) study was available in the fathead minnow. The highest nominal dose in this study resulted in marked reduction of F0 survival and F0 reproductive performance, with no reproductive effects seen at doses where impairments of growth and survival were observed. Multiple studies have measured brain ChE levels in fish following exposure to carbaryl, as well as other carbamate pesticides. For example, two studies with rainbow trout reported statistically significant decreases in brain ChE levels at aqueous carbaryl concentrations ≤ 0.25 mg/L. Based on previous studies, it can be concluded that carbaryl, at doses identified as levels demonstrating systemic toxicity in two fish reproductive studies, would be expected to inhibit ChE and is likely the primary mechanism driving reproductive and possible endocrine effects. In the FFLC study, concentrations that affected reproductive parameters also reduced survival, and the survival endpoint is thus protective of effects on reproduction, regardless of MoA. However, it can be concluded that the reproductive and endocrine findings in the FSTRA and the FFLC studies are secondary to ChE inhibition and systemic toxicity.

WE232
Metals are indispensable, they define the health status of mammals and wild life. Can they also be endocrine disruptors?
R. Cortvrindt, Arche consulting; V. VEROUGSTRAETE, Eurometaux / EHS; M. Vangheluwe, ARCHE
Within an organism, the regulation of the uptake and excretion of the exogenous

metals present several similarities with the internal production and metabolisation of hormones. They both have a complex equilibrium between their different constituents and can have complex non-monotonic dose response curves. The effect(s) are dependent on the actual internal concentration within the organism, their homeostasis can easily be disturbed by external influences, thereby eliciting detrimental effects within the respective organism or population. The most vulnerable life stages are the developing foetus and offspring, disruption of their endocrine or metal homeostasis can generate long-term effects that has its main impact during adult life or even in the next generation. The concerns regarding the presence of metals and endocrine disruptors in food, water or other environmental media, as well as the potential risks they pose to humans and wildlife have been growing in recent years. The European Commission has adopted the definition of endocrine disruption set by the WHO in 2002, and is in the process of revision of its legislation accordingly. However, for its implementation there is still an (open) discussion on the criteria that will be used to mark compounds as endocrine disruptors. We have performed a review of the available literature, to gather information to evaluate whether metals might be at risk for classification as endocrine disruptors. As a first approach, we focussed on review papers on metal(s) and endocrine disruption, to get a general insight in the topic. Thereafter literature was screened for endocrine (disruption) in relation to individual metals. Special attention was also given to papers focussing on ‘a metal’ in combination with environment and invertebrates. We came to the conclusion that it will not be an easy task to define whether or not a metal is an endocrine disruptor according to fixed criteria. Experimental studies to assess adverse endocrine activity of metals often use exposure levels in excess of those encountered in the different environmental matrices. The dosing often interferes within the natural balance of the different metals within the organism and the used apical endpoints cannot always be linked to an endocrine mode of action. We will summarise the state of play and knowledge on a number of metals and propose some ways forward.

WE233
SETAC Interest Group on Endocrine Disrupter Testing and Risk Assessment
A. Leopold, Independent Consultant/ Researcher / Calidris Environment BV

Risk assessment and management of waterbodies (ground, fresh, marine and drinking waters) (P)

WE234
Use of macroinvertebrates and fish to determine priority substances concentrations in Walloon Rivers: biota monitoring network and caging techniques.
D. Leroy, ISSeP / Ecotoxicology; P. Libert, Service Public de Wallonie SPW; A. Galloy, I. Hardy, M. Canisius, ISSeP; Y. Marneffe, Inst. Scientific de Service Public / Ecotoxicology Department
The European Water Framework Directive (WFD) aims to achieve and ensure a good quality status of water in each Member State. Most of the Environmental Quality Standards (EQSs) are defined for water itself. However standards have also been set in biota for some substances (Directive 2008/105/EC and Directive 2013/39/EU). This is the case of mercury, hexachlorobenzene (HCB), hexachlorobutadiene (HCBD), PAHs (benzo-a-pyrene and fluoranthene), Polybrominated diphenylethers (PBDEs), Perfluorooctane sulfonic acid and its derivatives (PFOs), dioxins and dioxin-like compounds, heptachlore, heptachlore epoxyde, hexabromocyclododecane (HBCDD), and dicofol. To answer these recommendations we developed methods to measure the concentration of these pollutants in freshwater biota sampled in Walloon Rivers. A preliminary study conducted from 2013 to 2015 on four fish species (*Leuciscus cephalus, Abramis brama, Cottus gobio* and *Barbatula barbatula*) and macroinvertebrates species (crustaceans and/or molluscs) on 54 sampling sites allowed us to implement a monitoring network on freshwater biota that began in 2016. This monitoring network counts 120 sites representatives of the Walloon hydrographic districts. These sites are sampled once every two years (60 sites sampled every year). Differences in contamination between the studied sites are discussed and obtained values are compared to EQSs. Sentinel species are not present in every sampling site. To overcome this problem, a caging technique with the crustacean *Gammarus pulex* has been developed. Gammarids coming from a clean site were acclimatized for two weeks in the laboratory and caged in one reference site and one polluted site. The experiment lasted one or two weeks to establish the time to equilibrium and was repeated 4 times in a year, once per season. Benzo-a-pyrene and fluoranthene were searched in these organisms after caging. Moreover, this caging technique has been used in 7 sites in 2016. Results are discussed.

WE235
Assessing contaminant risk at Areas of Concern in Lake Superior
N. Gandhi, University of Toronto / Dept of Earth Sciences; D. Jackson, University of Toronto / Ecology and Evolutionary Biology; S.P. Bhavsar, Ontario Ministry of the Environment and Climate Change
North American Great Lakes contain about 20% of the world’s fresh surface water. Historical industrial activities around the Great Lakes resulted in elevated levels of contaminants in various matrices including sediment and fish. In the 1980s, 43 local areas within the Great Lakes were designated as Areas of Concern (AOCs) mainly due to the contamination issue. Fourteen beneficial uses were defined to evaluate status of the AOCs. Assessing whether fish consumption beneficial use is still impaired or not remains an important step for delisting of the AOCs where this use was deemed impaired due to elevated contaminants. This presentation will discuss fish contaminant monitoring data for Lake Superior, which is the largest of the five Great Lakes, and investigate if two AOCs of the lake have recovered from the various risk assessment and management perspectives. The observations will be presented in light of various other factors including chemical mixtures that can contribute in understanding the environmental conditions. The findings of this study will help in addressing challenges involved in evaluating fish consumption beneficial use impairment at the AOCs and path forward towards a re-designation.

WE236
Beyond guidelines: the benefits of applying aquatic sediment toxicant weight-of-evidence assessment to environmental regulation
P.J. Leahy, EPA Victoria / Applied Sciences; V.J. Pettigrove, The University of Melbourne / Zoology; L. Metzeling, EPA Victoria / EPA Victoria
The current approach of comparing test results for chemicals in the environment to water and sediment quality guidelines is inadequate because the development of guidelines can never keep pace with the growth of new chemicals in the environment, and the potential toxicity of mixtures is unknown and cannot be predicted by comparison with individual guidelines. Weight-of-evidence assessment has much to offer environmental regulators who seek to move away from traditional assessment of chemical concentrations against guidelines to one based on environmental outcomes. Weight-of-evidence creates its own challenge for environmental regulators, as there is a need to make an objective overall decision on the acceptability of the weight-of-evidence assessment. A recently released aquatic sediment toxicant weight-of-assessment approach for Australia (Simpson and Batley, 2016) has been evaluated for application in environmental regulation. We have evaluated the proposed approach, which includes chemistry, toxicity, ecology, bioaccumulation and other approaches including biomarkers, and recommended that it be adopted within the environmental regulatory framework in Victoria for freshwater, estarine and marine environments. The approach allows for the scoring of different lines of evidence and provides for an overall risk rating. It integrates existing sediment quality and biological indicators with laboratory based whole sediment ecotoxicology. Importantly, it allows for the addition of new lines of evidence as they become accepted in the scientific literature. We present an evaluation of the approach using whole sediment ecotoxicity data collected from Victoria and conclude that a weight of evidence approach could provide a sensitive indicator of emerging toxicants and quantify the impact of complex chemical mixtures. **Reference:** Simpson, S. and Batley, G. (eds) (2016). Sediment quality assessment : a practical guide. CSIRO Publishing.

WE237
Development of a triad assessment method for brackish sediments in Flanders
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In sediment risk assessment, analysis of the pollutant concentrations is essential in determining the degree and nature of sediment contamination. However, chemical analyses provide no evidence of toxic effects or effects in situ. The Sediment Quality Triad method incorporates measures of various chemical parameters, toxicological effects and benthic community structure in view of conducting an integrated assessment of sediment quality. In framework of developing a triad assessment method for the quality evaluation of brackish sediments in Flanders, in 2015-2016 38 sediment samples were taken along the Scheldt estuary (Sea Scheldt (Flanders) and Western Scheldt (the Netherlands)) and other brackish aquatic systems in Flanders. For these samples, chemical parameters (e.g. metals and organic pollutants), toxicological effects and benthic community structure were assessed. In this study 3 bioassays were carried out to test their suitability for uptake in a quality triad method for brackish sediments as indicator of ecotoxicological effects. Two sediment contact bioassays with the polychaete worm *Hediste diversicolor* and the amphipod *Corophium volutator*, and a pore water test with the rotifer *Brachionus plicatilis* were performed to test the toxicity of the samples. For the sediment contact tests observed mortalities varied between 0%-33% for *H. diversicolor*, between 1%-22% for *Corophium volutator* and between 10%-43% for *B. plicatilis*. Control tests with reference sediment showed for the 3 tests mortalities lower than 10%. For the Scheldt estuary, the 3 tests showed significantly higher mortalities for sediments from the upper part of the estuary in the Sea Scheldt, which was characterized by higher levels of micropollutants, than for the lower part of the estuary (Western Scheldt). Based on a literature inventory of existing sediment quality standards, quality guidelines for chemical parameters for Flemish brackish sediments were formulated. For the biological component of the triad method, comprising an evaluation of the benthic invertebrate community, a separate evaluation method was included for brackish oligohaline more static water systems, and for brackish sediments in the different ecotopes of the Scheldt estuary.

For the latter the M-ABMI ('Multivariate AMBI', Bald et al., 2005; Muxika et al., 2007) and the Occurrence Intactness Index are tested for their suitability as biological index in a triad method for the evaluation of Scheldt sediments.

WE238

Ecological risk assessment of Lake Mondsee, Austria: ecotoxicological line of evidence

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Lake Mondsee is a recreational area in Austria for both bathing and water activities/sports. Additionally to the input of chemicals originated from tourism activities (e.g. sunscreen), this lake receives the discharge of a wastewater-treatment plant (WWTP), which may contribute to an increased ecological risk. An ecotoxicological line of evidence was addressed to assess the potential ecological risk of Lake Mondsee. Water and sediment samples from Lake Mondsee were collected in the summer of 2015 and spring of 2016. A nearby lake (Irrsee) was used as a reference site. The WWTP inflow and outflow (water samples), plus pre-thickening and thickened sludge (using chalk) from the WWTP were also collected. The following assays were carried out with water samples (filtered and non-filtered): i) 48-h chronic test with *Brachionus calyciflorus*; ii) Microtox® test with *Vibrio fischeri* (81.9% Basic test) (only non-filtered samples) and iii) 72-h growth inhibition test with *Raphidocelis subcapitata* (only 2016 spring samples). Sediment and sludge toxicity was assessed through: i) Microtox® test (Solid Phase Test) and ii) 6-day mortality and growth assessment with *Heterocypris incongruens* (only 2016 spring samples). The 2015 summer water samples, from both lakes (Mondsee and Irrsee) and the WWTP outflow revealed no toxicity: no luminescence inhibition was registered for *V. fischeri* and average population growth inhibition rate (%) of *B. calyciflorus* was below 26%. For the WWTP inflow, samples presented high toxicity to *B. calyciflorus* (EC_{50} of 60.8% and 87%, for non-filtered and filtered samples, respectively). Sediment and sludge samples were very toxic to *V. fischeri.* The 2016 spring samples results were similar to the previous ones: the average population growth inhibition rate (%) of *B. calyciflorus* was lower than 30% for most of the water samples and the Microtox® tests showed no toxicity for all water samples and high toxicity for all sediment and sludge samples. No toxicity was observed regarding growth inhibition of *R. subcapitata* (average growth inhibition below 12%). The results suggest that the WWTP is not causing a higher ecological risk at Lake Mondsee compared to Lake Irrsee, since water and sediment samples from both sites showed similar ranges of toxicity. Chemical analysis may clarify the causes of the high toxicity observed in the sediments. One hypothesis that could explain such results is the fact that these sediments are partly anoxic.

WE239

Multi-actor strategy for the management of an industrial effluent impacting a sensitive waterbody: use of bioassays to assess the efficiency of the management measures.

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Since many years, a highly polluted tannery effluent is impacting a very sensitive waterbody, the Pas-à-Wasmes watercourse. Despite expensive improvements in treatment carried out by the industrial operator, the toxic load as shown by a bioassay battery remained important for the receiving river (waterbody EL13R). The complementary management of the effluent by the urban treatment plant was not possible before and the industrial effluent was directly discharged into the river. In 2013, the main stakeholders (the industrial operator, the regional authority delivering permits, the operator of the urban waste water treatment plant of Pas-à-Wasmes and scientists - ISSeP and Cebedeau) began discussions in order to solve this problem and assess the efficiency of the decided management measures. The improvement of the pretreatment in the tannery and the tannin recycling allowed the urban waste water treatment plant to treat the totality of the industrial effluent without suffering adverse effects on its own treatment capacities. To assess the efficiency of the treatment, complementary to chemical analyses, we used a battery of short term and chronic bioassays with the bacteria *Vibrio fischeri*, the alga *Pseudokirchneriella subcapitata*, the rotifer *Brachionus calyciflorus* and the microcrustacea *Daphnia magna*. Priority List substances of the WFD and other pollutants discharged in significant quantities were also measured. The analyses were carried out on the effluent of the tannery before and after treatment by the urban waste water treatment plant and in the receiving waterbody, upstream and downstream from the effluent. Results show that the toxicity of the industrial effluent is strongly lowered after the treatment by the urban treatment plant and bioassays show that the receiving waterbody is really significantly less impacted than before. This management resulting from a multi-actor strategy induced an important improvement of the waterbody in a cost-effective way.

WE240

Non-linear relationship between estrogenic activity and sample dilution

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Endocrine-disrupting compounds pose a global threat to human health and the environment. There is a strong need for standardised and reliable methods evaluating the endocrine-disrupting potential of environmental samples with a special emphasis on estrogenic activity. Effect-based *in vitro* tests, using genetically modified yeast or mammalian cells as test organism, inherently capture the effects of the whole sample on the test organism. This also includes sample components that do not have a direct endocrine effect, but can affect endocrine activity through combinatorial effects. Here, we analysed estrogenic activity in different water samples (spiked surface water, spiked sewage effluent, municipal sewage influent and hospital sewage influent) at 6 different dilution levels ranging from the undiluted sample to a dilution level of 9.6, using the recombinant yeast bioassay Arxula Yeast Estrogen Screen (A-YES®). For each analysed sample dilution the 17β-estradiol equivalent concentration (EEQ) was calculated based on the 17β-estradiol (E2) calibration curve. Subsequently the dilution-adjusted EEQ ($EEQ_{adjusted}$) was calculated ($EEQ_{adjusted}$ = EEQ*sample dilution) and the relationship between sample dilution and $EEQ_{adjusted}$ was examined by weighted linear regression analysis. Results revealed no differences between $EEQ_{adjusted}$ across all dilution levels for the spiked surface water and spiked effluent samples, whereas $EEQ_{adjusted}$ of sewage influent and hospital sewage influent samples significantly increased with increasing dilution levels ($p < 0.01$ for both samples). Our results demonstrate that outcomes of effect-based *in vitro* tests highly depend on sample composition, *i.e.* sample dilution, prior to analysis. Risk assessment of waterbodies using effect-based *in vitro* tests depends on matrix interferences and is therefore not only sample- but also dilution/enrichment-specific. Thus, the phenomenon of sample-specific relationships between endocrine activity and sample dilution should be taken into consideration when implementing water quality legislations.

WE241

New insights on active biomonitoring using the freshwater amphipod Gammarus fossarum: various cases of impact assessment measuring micropollutants bioaccumulation and ecotoxicological effects.

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The degree of pollution of waterbodies is traditionally appraised by micropollutants research and quantification applied in sediment or in water. While this chemical approach can determine contamination of the targeted compounds in waterbodies, it is inadequate for assessing the bioavailability of chemical substances and toxic impact of aquatic environments (characterized by complex mixture of pollutants) which are currently proposed as complementary analysis. Because of the need of such diagnosis tools for public and private managers, substantial efforts have been made over the last 30 years to develop and apply innovating biological approaches for risk assessment. In this context, this presentation focuses on using a crustacean model, the freshwater amphipod *Gammarus fossarum*, to assess chemical contamination and toxicity. Our approach of active biomonitoring, based on calibration of "control" individuals (size, sex, population,…) which were exposed during 7 days directly in waterbodies (by caging methodology), is an integrative and relevant approach for field experiments. To demonstrate it, we will show how *in situ* bioassays bring complementary information to public and private managers in illustrating by various cases of study: waste water treatment plant, storm pillway, dam draining as well as industrial sites. During these field experiments, we finally assessed 1- level of bioavailability contamination for metallic and organic compounds in exposed gammarids and 2- ecotoxicological effects on global parameters (survival, feeding rate, reprotoxicity) and on specific parameters (acetylcholinesterase activity, endocrine disruption). For a reliable data interpretation, we proposed threshold values for bioaccumulated concentrations and biological responses. The general purpose is to highlight the operational, cost-effective and reliable character of active monitoring using gammarids for a large scale deployment in waterbodies.

WE242

Study on the biochemical markers of Dreissena polymorpha and Coregonus lavaretus from two freshwater Austrian lakes: a spatio-temporal monitoring approach

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The uncontrolled release of potentially contaminated wastewaters constitutes an increasingly concern for the health of freshwater ecosystems, which threatens to impair the biodiversity resilience and the ecosystem services (*e.g.* fishing, bathing, water tourism) on which human wellbeing relies on. In this study it was aimed to assess if the discharge of a wastewater treatment plant (WWTP) effluent caused biochemical alterations in natural populations of the European whitefish *Coregonus lavaretus* and of the zebra mussel *Dreissena polymorpha*. To attain this goal, individuals of *C. lavaretus* and of *D. polymorpha* were collected in two freshwater Austrian lakes: Lake Mondsee that receives the discharge of a WWTP, and Lake Irrsee (without the influence of any WWTP effluent). Samplings were carried out during three field campaigns (Summer and Winter, 2015-only for fish; and Spring, 2016-for fish and mussels). The following set of biochemical markers was evaluated in fish organs and in the whole body of mussels: (i) lipid peroxidation (LPO); (ii) oxidative stress (catalase and glutathione *S*-transferase); (iii) neurotransmission (cholinesterase); and (iv) energetic metabolism (lactate dehydrogenase; LDH). In general, slight differences were observed in the biochemical markers between the two lakes for the two studied species. However, a significant and seasonally-independent difference of some biochemical markers was observed for fish, especially in muscle LDH (lower in Lake Mondsee) and liver LPO (lower in Lake Irrsee). It is suggested that these results may be due to natural differences in the physical, chemical and biological characteristics of these to lakes. Further physical and chemical characterization of the lakes' water is still needed to better understand these results. \n *Keywords*: biochemical markers, freshwater ecosystems, risk assessment, wastewater effluent

WE243
Which Environmental threshold values to evaluate the Good Environmental Status of the European Union marine waters in the context of the Marine Strategy Framework Directive?
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The Marine Strategy Framework Directive (MSFD) aims to achieve Good Environmental Status of the EU's marine water by 2020 and to protect the resource upon which marine-related economic and social activities depend. The Directive lists four European regions: the Baltic Sea, the North-East Atalntic Ocean, the Mediterranean Sea and the Black Sea located within the geographical boundaries of the existing Regional Sea Conventions. France is involved in four sub-regions: The Channel-North Sea, Celtic seas, Bay of Biscay and Western Mediterranean. In order to achieve GES by 2020, each Member State is required to develop a strategy for its marine waters with definition and implementation of monitoring and measures programs. GES is defined using 11 descriptors. This study focuses on descriptor 8 on "contaminants". GES under this descriptor is reached when "contaminants are at level not giving rise to pollution effects". Environmental chemical threshold values have therefore to be defined. Environmental threshold values do already exist for several MSFD priority contaminants used within other contexts. The Water Framework Directive (WFD) sets the strategy against the chemical pollution of surface water bodies including transitional water bodies. To determine the overall quality of a water body, Environmental Quality Standards (EQS) are used. OSPAR sets the objective of preventing pollution of the maritime area by continuously reducing discgarges, emissions and losses of hazardous substances, with ultimate aim of achieving concentrations in the marine environment near background values for naturally occuring substances and close to zero for man-made synthetic substances (background assessment criteria (BAC)). Environemental Assessment Criteria (EAC) are assessment tools intended to represent the contaminant concentration below which no chronic effects are expected to occur in marine species. In order to determine which environemental threshold should be used for the implementation of MSFD (EQS, EAC, other threshold), a comparaison is done between the metodology used to determine EQS and EAC. This allows to highlight the main differences existing between both approaches and to explain how different values are obtained for one contaminant. This work focuses on two contaminants for which both EQS and EAC values are available on molluscs.

WE244
A novel evaluation of the loads of nitrate and phosphate in rivers by using passive samplers and compact continuous samplers
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Nitrogen and phosphorous are major nutrients to various plants. However, nitrate (NO_3^-) and phosphate (PO_4^{3-}) are notorious in rivers, lakes, by and water reservoirs because they can cause eutrophication in various countries. In Japan, some closed water areas are so eutrophicated that it is important to control and estimate the load of nitrate or phosphate from rivers. The monitoring of their load is generally performed by grab sampling once a month. Occasionally, Grab samplings also must be conducted in runoff events because concentrations of nitrate and phosphate in river water, which contains high concentration of particulate matters, are remarkably higher than those in fine days. Continuous samplings should be needed to estimate their annual loads precisely for feasible countermeasures to eutrophication. Actually, many Japanese scientists discuss large variations of these loads basic units and also need basic loads with a smaller margin of error. In this study, a passive sampling technique is applied to estimate basic units of nitrate and phosphate in dissolved phase in rivers. The Chemcatchers with Empore disks were deployed in rivers for several weeks in order to estimate their time-weighted average concentrations during the deployment. The depth is also measured continuously in the deployment to calculate flow rates. A compact continuous sampler developed in our laboratory is applied to concentrate particulate matters in surface water during the period. This sampler "GRAVE" can accumulate particulate matters at a constant rate automatically. The masses of nitrate and phosphate in particulate phase are analyzed after the recovery of samplers. A grab sampling method, which is an usual method to measure the loads, is also conducted to compare loads and basic units from passive and GRAVE method. This novel method has great potential possibility to estimate loads of various pollutants such as metals, hydrophilic and hydrophobic chemicals, microplastics, radioactive caesium, which was already applied in my laboratory, and other emerging contaminants.

WE245
Submerged aquatic vegetation as an indicator and focus of risk assessment and management in a shallow hypereutrophic lake
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Lake Mattamuskeet is a 16,200 ha, shallow (< 1m) lake located on the eastern coast of North Carolina, USA. Lake Mattamuskeet is the centerpiece of Mattamuskeet National Wildlife Refuge, established to maintain and promote habitats for migratory birds, specifically wintering waterfowl. The lake recently experienced a reduction in water clarity and macrophyte beds (also referred to as submerged aquatic vegetation, or SAV) and increases in phytoplankton, harmful algal blooms, and cyanotoxin production. To facilitate restoration, we focused risk assessment problem formulation on SAV survival and recruitment. Because of SAV's importance to management goals and ecological relevance as a foundation of the aquatic food web, this one assessment endpoint and indicator both galvanized stakeholder support and simplified risk assessment. We adopted a conceptual model of SAV stressors to provide a visual representation of potential limiting factors, including water clarity, nutrients, sediment, and pesticides and the factors that influence each of these. We gathered and expanded upon existing datasets to document significantly increasing trends in surface water nitrogen, phosphorus, turbidity, suspended sediments, chlorophyll *a*, pH, and decreasing SAV coverage between the late 1980s and more recent 2013-2015 SAV surveys. Through comparison to established benchmarks, we also documented that current water quality conditions (2012-2015) are not conducive to SAV survival and recovery and, in some cases, do not meet North Carolina water quality standards for the protection of aquatic life. Moving forward, SAV continues to serve as our indicator species for lake health in guiding monitoring, research, and management efforts now focused on the restoration of SAV. Although the biology and ecology of shallow lakes is complex, we observed that problem formulation, conceptual model development, and assessment and measurement endpoint selection focused solely on SAV provided the management value and scientific rigor to effectively move stakeholders from large uncertainties to cooperative restoration.

WE246
EU Regulatory requirements for surfactants - challenges and opportunities
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Surfactants are surface active substances with various properties depending on their speciation. They have the ability to lower the surface tension, micelle formation, and even act with antimicrobial properties, and hence they are widely used in consumer products, such as disinfectants, demulsifier, emulsifiers, laundry and home cleaning products. However, in order to ensure environmental safety, surfactants are subject to a number of regulatory restrictions. In particular, biodegradability potential of surfactants is a crucial consideration governing use in detergents. Here we present an overview of regulatory requirements in the European Union (EU) and the reasons behind them. The EU Detergents Regulation (EC) No. 648/2004 restricts the use of surfactants in European detergents based on their biodegradability. EU Flower and SWAN Ecolabelling Schemes demand aerobic ready biodegradability and anaerobic biodegradability to provide confidence that surfactants used in Ecolabelled products are safe for the environment. These regulatory restrictions give an opportunity to promote the use of environmentally friendly surfactants by formulators, and for the companies to improve their global portfolio of surfactants.

WE247
Concentrations of bisphenol A in European surface waters: Estimating summary statistics used in deterministic and distributional risk analyses
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Bisphenol A (BPA) is mainly used as a chemical intermediate to produce polycarbonate plastics and epoxy resins. During the entire lifecycle of BPA which comprises manufacturing, processing, use, and disposal of polycarbonate and epoxy resin products, as well as other products, traces of BPA may enter surface waters, mainly via wastewater treatment plants or landfills. BPA is frequently included as an analyte in surface water sampling programs. To gain an overview on the available data, concentrations of BPA in European fresh and marine waters

were compiled from published scientific literature, governmental reports, and the NORMAN network`s EMPODAT database. The dataset now contains ca. 30,000 entries of data, collected from 1996 to 2014, from all sources. Three areas for consideration were identified that needed to be addressed to enable calculation of summary statistics: First, most of the measured values were actually below a limit of detection for a study and detection limits varied over several orders of magnitude across all studies. The Kaplan-Meier method was used to estimate concentrations below detection limits from the distribution of values above these detection limits in studies with a lower detection limit. Second, many studies only reported summary statistics such as means or ranges. When the number of samples was known, values were imputed for that number of samples so that the summary statistics of the imputed concentrations were identical to the summary statistics reported and the distribution of the imputed concentrations approximated a log-normal distribution. Third, many sampling campaigns collected samples over broad areas, while others collected multiple samples at few locations thus requiring the use of weighting factors such that each location carried the same weight in calculating summary statistics. For Europe, a total of 3,675 freshwater and 456 marine water weighted observations are available. For freshwater locations, 30 % were below a detection limit (varying detection limits). Median and upper 95th centile concentrations for the full distribution of weighted observations were 0.029 and 0.30 μg/L. For marine locations, 39 % were below a detection limit. Median and upper 95th centile concentrations were 0.007 and 0.15 μg/L. The distribution of weighted observations of BPA will be compared to the distribution of aquatic ecotoxicity data for freshwater and marine organisms.

WE248

Single substance and mixture toxicity of emerging polar micropollutants detected in the marine environment

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The European Union's Marine Strategy Framework Directive (MSFD) adopted in 2008 aims to achieve Good Environmental Status (GES) of the EU's marine waters by 2020 and to protect the resource base upon which marine-related economic and social activities depend. Good Environmental Status is defined as: "The environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive." In practice, involved countries should aim for maintenance of biodiversity, healthy populations of commercial marine fish species, food webs ensuring long-term abundance and reproduction, and no effects caused by contaminant concentrations. The NewSTHEPS project addresses the current fundamental scientific and methodological issues related to the implementation of GES of the marine environment by development of novel procedures for comprehensive environmental monitoring and risk assessment of a broad set of both priority and emerging contaminants in the marine environment. In March 2016, an explorative sampling campaign and chemical monitoring based on SPE-LC-Orbitrap-HRMS was performed in one Belgian harbour and at one open sea location in the Belgian part of the North Sea; and several personal care products, pesticides and pharmaceuticals were detected at both sampling sites. It was our goal to determine chronic effect concentrations for some of the detected compounds to fill toxicity data gaps of emerging polar chemicals for marine organisms. Thus, we chose 2 pesticides (chloridazon and pirimicarb) and 2 pharmaceuticals (carbamazepine and sulfamethoxazole) based on detection frequency, lack of marine ecotoxicity data and physico-chemical properties. The selected substances were tested individually and as mixtures in a 72h growth inhibition test with the marine diatom algae *Phaeodactylum tricornutum* (according to the ISO guideline 10253) and effect concentrations were determined. The results will be presented and discussed in a context of marine chemical mixture risk assessment. **Acknowledgments** The authors like to acknowledge the Belgian Science Policy (BELSPO) for funding the NewSTHEPS project (BR/143/A2/NEWSTHEPS; www.newstheps.be). The financial support from the Hercules Foundation (Flemish Government; AUGE/11/016) and from the Ghent University Special Research Fund (01B07512) is acknowledged for the UHPLC-Q-Exactive^{TM} and the automated SPE equipment, respectively.

WE249

Development of a rapid screening methods for pathogenic bacteria by using chemical contaminants in Olympic Triathlon swimming courses at Tokyo bay

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The coastal areas are important for sound ecosystems and economic activities such as fishing industries and recreations. Especially, Triathlon games will be held at coastal areas in Tokyo bay Odaiba marine park) in 2020 Tokyo Olympic game. However, surface water in swimming areas for triathlon is contaminated by chemicals and pathogens in combined sewage overflows (CSOs) from Kanto metropolitan area. Occasionally, concentrations of *Escherichia coli* **(***E. coli*) exceed the water quality standards recommended by International Triathlon Union. Therefore, effective countermeasures should be performed by 2020 Olympic games and water quality should be monitored during the games. But, it takes about one day to measure the concentrations of these bacteria by general methods. Moreover, these concentrations fluctuate daily by weather condition. As a result, it is difficult to evaluate these concentrations and human risk on the day of the games. Adding to that, CSOs also carry chemical pollutants, especially fecal sterols. Though these contaminants do not have adverse effects on athletes, previous reports discussed their correlations to *E. coli.* This study will demonstrate some simple indicators for *E. coli* at swimming courses in Tokyo Bay. The concentrations of these pathogenic bacteria are monitored with simple water qualities such as transparency, suspended matters (SS), total organic carbons (TOCs), caffeine and crotamiton. Transparency and SS are influenced intensely by algae bloom in the bay. However, a new TOC analyzer could demonstrate water contamination near coastal areas and could reveal that dissolved organic carbon concentrations have a strong relationship to *E. coli* concentrations at Tokyo bay. This may be caused by various unknown fecal pollutants in dissolved phase. Caffeine and crotamiton are reported to be useful indicators for contamination by sewage water. This study also revealed their relation to *E. coli* concentrations. A battery of TOCs and several chemical pollutants has potential about simple and rapid indicators for pathogenic contamination and human risk at swimming courses. We also develop a smartphone software application that allows for on-site estimation of water quality as well as risk evaluation for swimmers in Odaiba marine park. Furthermore, we try to establish novel water monitoring and swimmers risk management procedures (including smartphone software) for recreational water area near metropolitan area.

WE250

Spatial analysis of human health risk through drinking groundwater in Taiwan's Lanyang Plain

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Chronic arsenic (As) exposure continues to be public health problem of major concern worldwide. A groundwater quality survey has revealed that part of groundwater in northeastern Taiwan's Lanyang Plain is clearly contaminated with the measured As concentration in excess of the harmful level recommended by the WHO of 10 μg/L. Efforts for assessing the health risk associated with the intake of As through the contaminated drinking groundwater are required as part of the important work for health risk management. Considering that the conventional approach to conducting human health risk assessment may be insufficient, this study performs a spatial analysis of the health risk associated with ingesting As through drinking groundwater in the Lanyang Plain. First, the spatial distribution of the As occurrence is analyzed by using the geostatistical interpolation method. The health risk assessment based on the hazard quotient (HQ) and target cancer risk (TR) established by the U.S. Environmental Protection Agency is then spatially evaluated and mapped based on the spatially estimated concentration. Ultimately, a map that delineates areas with high TR values and high population densities is provided. The findings in this study provide a basis for improving the decision-making process of the health risk management.

WE251

A milestone on the way to a "whole" water Certified Reference Material: ERM-CA100, PAHs in surface water

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Polycyclic Aromatic Hydrocarbons (PAHs) are among the Priority Substances (PS) identified by the Water Framework Directive (WFD) for which the EU Member States shall apply Environmental Quality Standards (EQS) [1] towards the achievement of good surface water chemical status. Directive 2009/90/EC [2] establishes minimum performance criteria for the analytical methods used in the WFD monitoring and requires the laboratories to demonstrate their competence through the use of Reference Materials (RMs) that are representative of collected samples. In the case of organic PS, the water EQS are expressed as total concentrations in the whole (non-filtered) water sample given the high hydrophobicity of many of these molecules. This requirement translates in a significant challenge for the production of CRMs which should mimic surface water, containing variable amounts of dissolved and/or suspended organic matter. This project concerns the production of ERM-CA100, a water RM containing humic acids (HAs), certified for the concentrations of naphthalene, anthracene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and indeno[1,2,3-cd]pyrene. The CRM consists of 3 items (water, HAs solution, PAH solution) to be combined at the laboratory's premises following an easy-to-apply reconstitution protocol. Homogeneity and stability of the CRM were assessed: their contributions to the uncertainty of the certified values were estimated between 1.7 to 4.7 % and 1.4 to 8.6 %, respectively, depending on the PAH congener. The certified values, determined through an interlaboratory comparison of expert laboratories, were assigned to seven PAHs between 29 to 104 ng/L (except naphthalene's value of 1.21 μg/L). The main analytical challenge was the control over the PAHs adsorption onto the HAs in the water matrix [3]. This is the first CRM approaching a whole water sample, as requested by the WFD, through the addition of HAs to simulate organic matter. The CRM will be useful for assessing the performance of the analytical methods applied in the mandatory monitoring of water bodies under the WFD. [1] Directive 2013/39/EU Off J. Eur Union L 226 119. [2] Directive 2009/90/EC Off J Eur Union L 201, 36. [3] J.Van de Kreeke, B.de la Calle, A.Held, O.Bercaru, M. Ricci, P.Shegunova, P.Taylor. 2010. Trends Anal Chem 29 (8) 928-937. The authors thank Z.Spalt, A.Held and H.Emons for supporting the project and the RMs Processing Team of JRC-Geel for technical contribution.

WE252

Derivation of Environmental Quality Standards for highly sorptive organic substances according to the EU Technical Guidance: A case-study for Deltamethrin

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Deltamethrin is a type-II pyrethroid known as a broad spectrum insecticide. Due to its very high adsorption capacity (K_{oc}= 10 240 000 ml/g; logK_{ow} = 6.4 – 6.99), it rapidly dissipates from water by sorption to suspended particles, biota or the sediment, thus reducing its bioavailability in aquatic systems. Deriving Environmental Quality Standards (EQSs) for such highly sorptive substances is challenging. This presentation examines how the EU Technical Guidance for deriving EQSs based on annual average and maximum acceptable concentration (AA-EQS and MAC-EQS, respectively) can be used to determine reliable realistic values for organic substances with high adsorption capacity, using deltamethrin as a case-study. Ecotoxicological data on deltamethrin and some of its formulations were retrieved from GLP and non-GLP studies. They were all checked for quality and reliability. Only acute and chronic endpoints expressed by reference to mean measured concentrations were considered as they better reflect the actual exposure conditions as compared to nominal concentrations. EQS derivation was based on the deterministic approach, as neither the probabilistic approach, nor the use of mesocosm data were applicable. For pelagic organisms, acute endpoints on 13 species belonging to fish, crustaceans, insects, mollusks and algae were used for MAC-EQS derivation. The AA-EQS was based on chronic data on 13 species from 5 taxonomic groups (fish, crustaceans, insects, algae, macrophytes). Freshwater and saltwater species were pooled since there were no statistically significant difference between the two data sets. For sediment organisms, the acute and chronic data sets were based on 4 species, and the endpoints were normalised to 5% organic carbon before calculation of the EQS. For substances with logK_{ow} > 6, the Guidance Document recommends correcting the initially derived EQS to account for the concentration adsorbed on suspended particles. The impact of such a correction on deltamethrin EQS was very significant. Deltamethrin AA-EQS and MAC-EQS were 0.12 and 0.28 ng/L, respectively, for freshwater. They were 4- and 20-fold lower, respectively, for saltwater. Lessons learned from the use of the EC Guidance Document for deriving deltamethrin EQSs are that, for highly sorptive organic substances, endpoints given as nominal concentrations are not reliable, and measured concentrations are required. In addition, considering the substance bioavailibilty is of primary importance.

WE253

A framework to assess the suitability of water quality standard values derived in different global jurisdictions as alternative water quality benchmarks for Canada

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Many regulatory authorities and institutions from different global jurisdictions (e.g. EU, North America, Australia and New Zealand) develop water quality standard concentrations for chemicals, i.e. the concentration of a substance in water below which no, or an acceptably low, degree of environmental hazard is predicted to occur. However, the specific procedures and methodologies employed by each jurisdiction in deriving their water quality standards can vary considerably, and this can often lead to significantly different 'safe' concentrations being applied for the same substance. The Canadian Council of Ministers of the Environment (CCME) develops Canadian Water Quality Guidelines (CWQGs) as a tool to provide credible scientific guidance on long-term no-effect and short-term impact concentrations in the aquatic environment. These are derived using the CCME Protocol for the Derivation of Water Quality Guidelines for the Protection of Aquatic Life (PAL) (CCME 2007). The CWQGs, and the CCME PAL Protocol itself, are well-respected both in Canada and internationally, and form the basis of environmental protection objectives with respect to chemical contamination in the aquatic environment in Canada. However, CWQGs can take considerable time and resources to develop, and consequently there are chemicals with the potential to impact Canadian waters for which CWQGs have not been derived. A framework has therefore been developed which assesses if water quality standard values derived by other jurisdictions, using alternative methodologies, are suitable as Alternative Water Quality Benchmarks (AWQBs) for Canadian waters. This Alternative Water Quality Parameter Assessment (AWQPA) framework provides procedures for the assessment and selection of the most suitable AWQB, as well as guidance on the use of non-AWQBs. The application of the AWQPA framework should therefore allow CCME to assess the applicability of guidelines developed by other jurisdictions to Canadian waters and to benefit from work on water quality guidelines conducted in other jurisdictions to strengthen protection of the aquatic environment in Canada. This poster will present the AWQPA framework, illustrate its application for selected substances, and highlight how the framework could be adapted for use in other jurisdictions who wish to fill gaps in water quality standard development in the most efficient manner, benefitting from work undertaken elsewhere.

WE254

INTCATCH: Development and application of novel, integrated tools for monitoring and managing catchments

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European citizens have a water environment to be proud of. In general, they can safely drink water from the tap and they have access to thousands of rivers, lakes and coastal waters for recreation. However, despite the first cycle of the Water Framework Directive implementation (2009 - 2012) costing at least €120 Billion^[i], many water bodies are still not meeting their environmental objectives. This is acting as a barrier to sustainable growth and improving quality of life in many parts of Europe. Environmental regulators have overseen the improvement to the water environment, taking us from the acute lethal, highly polluting point source discharges of the 1960s and 70s that significantly influenced our rivers and lakes. However, existing monitoring schemes run by regulatory agencies focus on classification, collecting samples and returning to laboratories for analysis. Although such sampling and analysis gives the widest range of measurements, it is time consuming, slow and labour intensive. These characteristics make it an expensive operation, and significantly, this approach doesn't deliver data to meet the needs of the wide range of stakeholders that are expected to be engaged in improving the water environment, as consultees and actors. So what needs to change? INTCATCH is a Horizon 2020-funded programme that aims to instigate a paradigm shift in the monitoring and management of river and lake water quality, by bringing together, validating and exploiting a range of innovative monitoring tools for river and lake water quality into a single efficient and replicable business model for water quality monitoring that engages the widest set of stakeholders and will be fit for European waters in the period 2020-2050. Working from the catchment-based approach, the project proposes new strategies 'taking the laboratory to the field', focused on a enabling a range of end users to use the many new technologies now available. These include smartphone apps, simple test kits, to fixed, and mobile stations such as radio controlled and autonomous boats that will be able to provide real time data for important parameters, that delivers the information needed to take the next step in catchment management to manage diffuse and ephemeral pollution. The poster display will be interactive and we aim to learn from delegates about their thinking around 'what are the problems we want to understand' to then 'what are the most effective ways of gaining that information'. <br clear="all" />

WE255

Assessing and valuing environmental damage and compensation in UK water bodies

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The UK has recently amended its Environmental Permitting Regulations to make provision for the regulator to accept an enforcement undertaking (EU) in the event of a non-compliance or pollution event associated with a permitted activity. This is a natural extension of civil sanctions, allowing operators to take action to remedy, compensate and improve the environment where harm has occurred. The approach to the evaluation of harm and compensation is in line with the European Environmental Liability (2004/35/EC) and Water Framework Directives. Such offences are usually accidental, but may result in serious harm and attract adverse publicity for the polluter. When the regulator accepts an enforcement undertaking, a number of benefits are offered for operators, the regulator, the public and the environment over criminal prosecution. This presentation aims to present case studies and lessons learned from water pollution incidents and describes how damage has been evaluated and compensation action accepted to ensure approriate remedy to the water environment. A brief overview of the enforcement undertaking process and its goals will be provided in the presentation, but the focus will be on the case studies. All of the cases to date have involved rapid releases of chemical pollution into surface (and ground) water bodies, so called 'pulse events', that have passed through the system into larger water bodies. Emphasis will be placed on the methodology used to quantify and value the damage caused. This has included best practice ecological risk assessment followed by habitat equivalency analysis to place a monetary value on the damage. The lessons learned will reflect upon the importance of establishing an accurate baseline condition and the ability to attribute cause and effect.

WE256

A source apportionment model for regulated chemicals
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The Water Framework Directive requires authorities to ensure no deterioration in the ecological status of water bodies, and that those not currently at good ecological status are restored. In order to plan measures to improve water quality it is essential to understand the sources of a contaminant to the water body of interest. Through an understanding of inputs from different sectors Regulators can put in place measures to improve water quality. This is, however, challenging due to a poor understanding of the sources, mobility and fate of many chemicals. The UK Water Industry and Regulators have recently collaborated to develop the Source Apportionment GIS (SAGIS) model, a decision supporting system that utilises a stochastic water quality model in combination with national data on pollutant sources and characteristics, effluent and river water quality, hydrology and land-use. Outputs from the model include source apportioned estimates of in-river chemical concentrations at a highly resolved spatial scale (1km intervals), as well as an inventory of chemical emissions by contributing sector. The modelling framework therefore satisfies a number of critical needs: Providing Regulators with a tool to quantify and report on the sources of chemicals. Providing the Water Industry with a tool to assist in identifying cost-effective ways of asset management to deliver water quality objectives. Providing a basis upon which to plan monitoring strategies. Providing a research tool to evaluate risk for novel and/or unregulated chemicals. This poster summarises the structure, mode of operation, limitations, calibration and 'goodness-of-fit' criteria of the model. A selection of outputs and findings from recent studies on a number of pollutants are provided.

Clean circular economy: recycling while eliminating legacy toxics (P)

WE257
How far does CENELEC EN 50625 contribute to a clean circular economy? The case of WEEE plastics recycling
R. Widmer, H. Böni, Empa Swiss Federal Laboratories for Materials Science and Technology / Technology and Society Lab; A. Haarman, EMPA Technology & Society Lab / Technology & Society Lab; P. Wäger, EMPA / Technology and Society Lab
More than two decades of experience with waste electrical and electronic equipment (WEEE) take-back and recycling systems have revealed persisting issues regarding toxic substances such as polybrominated flame retardants in plastics or polychlorinated biphenyls and electrolytes in electrochemical cells (capacitors and/or batteries). Since the establishment of the first European WEEE take back and recycling system in Switzerland in the early 90's of the last century, processing and legal requirements have tried to address this issue, with the new European norm CENELEC EN 50625 series as the most recent development in this regard. Based on our extensive experience in monitoring and evaluating the performance of the Swiss WEEE take-back and recycling systems, we will in our contribution discuss how far EN 50625 improves the sustainable handling of plastic fractions originating from the treatment of WEEE. Our main focus will be on how potent EN 50625 is in view of the elimination and/or isolation of contaminants in view of a closed loop recycling.

WE258
Challenges on the way to a non-toxic environment
F. van Broekhuizen, National Institute for Public Health and the Environment RIVM; M. Janssen, Nat. Inst. Publ. Health Environ. / Centre for Safety of Substances and Products; R. Luit, P. Wassenaar, National Institute for Public Health and the Environment RIVM
Plastics are an indispensible part of our economy. Its use has increased 20-fold in the last 50 years. Most of these plastics are being discarded after use, but there is increasing public request for recycling. These plastics though, may contain hazardous substances that will end up in new products after recycling. Challenges related to this legacy in terms of legislation, detection and separation of waste streams in the Netherlands were studied through interviews with representatives of waste treatment companies and end users of recyclate with a specific focus on treatment of waste containing POPs or substances of very high concern (SVHCs) under the REACH Regulation. It was found that waste treatment takes place within a network of companies operating at European level and depends on the type of plastic, its application and market demand and the expertise of the company. It was indicated that recovery of plastics containing SVHCs is most effective when sorting the waste stream straight after waste collection. Once the plastic polymer is mixed and grinded it will be much more difficult to separate out the SVHC containing material. In fact, dilution of the SVHC containing material will occur during mixing and grinding. Early separation of waste streams is experienced as a challenge because of the high variation and difficulty to indentify SVHC containing materials instantly and on site. The interviews further indicated uncertainties in legal obligations with regard to the End of Waste criteria and future SVHCs affected the recyling industry. Also, economic considerations played a mayor role in materials recycling and early separation of waste streams. Economic or legal incentives were suggested as possible instruments to improve the recovery of SVHC containing plastic waste to allow for targeted treatment. Targeted treatment should distinguish between recyclate containing POPs or SVHCs that may be reused safely and waste streams that can no longer. Hereby, negative impact of such substances on human health and the environment from recycling and reuse should be balanced against the possible pro's and con's of virgin material production.

Do we have the right tools to identify emerging hazards and risks? (P)

WE259
Classification & Labeling of Human Health and Environmental Hazards for Chemicals: Keeping a Global Perspective
C. Terry, Dow AgroSciences LLC; G. Meregalli, Dow Agrosciences; D. Juberg, Dow AgroSciences LLC
Introduction: There are different classification and labelling (C&L) systems and responsible bodies for chemicals across the globe. Many jurisdictions have adopted the United Nations Globally Harmonized System (UN GHS), either fully or with amendments. For example, in Europe, GHS has been implemented, with some alterations, as the classification, labelling and packaging of substances and mixtures (CLP) Regulation (EC) No. 1272/2008. The EU CLP is often perceived as one of the most conservative systems globally. Is this perception supported by data? **Methods:** An analysis was undertaken of the now 200+ chemicals whose harmonised C&L has been agreed by RAC, hereafter called the 'base set'. RAC CLP classification recommendations (those evaluated on or before 3 May 2016) were compared to initial proposals from the dossier submitter. This covered >800 individual classifications across the human health and environmental hazard categories. A statistical analysis of the agreements and differences was conducted. This 'base set' was then compared to relevant opinions from other expert bodies (European Food Safety Authority (EFSA), International Agency for Research on Cancer (IARC), US Environmental Protection Agency, and JMPR opinions). The conclusions of all expert groups were tabulated and compared. **Results:** Of the 'base set' of ECHA RAC recommendations (n=202), 74 had also been reviewed by EFSA, 26 by IARC, 34 by JMPR, and 25 by the US EPA. RAC agreed with the majority (78%) of the decisions on harmonised C&L proposed by EU MS/Industry. In 10% of classifications, RAC proposed a more severe C&L compared to the dossier submitter, and a less severe classification in 7% of cases. Other differences (5%) were not a reflection of disagreements with regards to severity. Besides respiratory sensitisation (100% agreement, but with only a single proposed classification), the highest level of concordance (99%) between opinions from RAC and dossier submitters was for having acute effects on the aquatic environment (Aquatic Acute). When the global classifications for each chemical was compared, there were significant differences. **Conclusions:** In conclusion, it is clear that when considering the global safety profile and hazard classification of a chemical, it is essential to consider all available information from various sources, and not to restrict consideration to only the jurisdiction/sector of interest.

Cost effective and ecological relevant testing using invertebrate species: new insights for environmental risk assessment (P)

TH001
Adverse effects of methylmercury (MeHg) on life parameters, antioxidative system, and MAPK signal pathways in the copepod Tigriopus japonicus
Y. Lee, J. Lee, Sungkyunkwan University
Among the various form of mercury in the aquatic environment, methylmercury (MeHg) is known to have the highest toxicity and easily biomagnified to higher trophic level organisms resulting up to 10,000-100,000 times higher accumulation in high-trophic orgasnisms (e.g., fish) than the concentration in the ambient water column. MeHg is known to exhibit various toxicities such as neurotoxicity, cytotoxicity, and developmental toxicity, and they are closely linked to generation of reactive oxygen species (ROS). Thus, in the present study, to evaluate the MeHg-induced oxidative stress in the aquatic invertebrate, the marine copepod *Tigriopus japonicus* was exposed to 1, 10, 100, 500, and 1,000 ng/L of MeHg. *T. japonicus* play a prominent role in energy transfer in the aquatic food chain and have ability to carry pollutants to higher trophic levels by digestion and accumulation, thereby probably the most suitable species to understand the potential impact of MeHg on the aquatic ecosystem. As a result, the level of ROS was induced in the concentration-dependent manner of MeHg, and subsequently the antioxidant enzymes including glutathione S-transferase (GST), glutathione reductase (GR), and glutathione peroxidase (GPx) was activated. In contrast, the activity of superoxide dismutase (SOD) was decreased with the negative correlation with the concentration of MeHg, suggesting that SOD have differently regulatory mechanism in response to MeHg exposure. The transcription level of antioxidant genes was up-regulated with concentration-dependency of MeHg. In the case of *GPx*, it seems to likely have post-transcriptional regulatory mechanism as their transcription level and the activity have shown the negative correlation. In addition, activation of ERK and JNK were observed at 1, 10, 100, and 500 ng/L of MeHg exposure, suggesting extracellular signal–regulated kinases (ERK) and c-Jun N-terminal kinases (JNK) on mitogen-activated protein kinases (MAPKs) pathway

are involved in signal transduction leading to oxidative stress response. This study will provide in-depth understanding to toxicity of MeHg on aquatic invertebrates.

TH002
Corbicula fluminea gene expression modulated by nCeO2 exposure and salinity
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Nanotechnology interest has considerably grown during last decades and nanoproducts are now part of our everyday life. Nanoparticle (NP) concentrations in natural environment are not yet known but releases during their life cycle are to consider. NP harmful effects have already been demonstrated on freshwater and marine species but their potential toxicity at more realistic low exposure concentration is still not well studied. Cerium dioxide nanoparticles ($nCeO_2$) are listed in OECD priority list of manufactured nanomaterials for their assessment because of their large production making them available for widespread uses in daily products. They are used in different fields such as automotive or optic. Several studies highlighted that $nCeO_2$ impact organism's physiology by affecting oxidative defenses and cellular membrane stability while gene expression studies remain still scarce and especially among non-sequenced organisms.\nThe aim of the present work was to evaluate gene expression disturbance occurring in *C. fluminea*, a widespread freshwater bivalve, exposed during 28 days under indoor mesocosm to multistress conditions. Two salinities (1.5 and 15 PSU) were applied to cover a range of salinity from the continent to the estuaries and three NPs were selected in order to have both standardized and commercial NPs but also to mimic changes occurring after NP uses. Two studies were done on variation of 12 gene expression by RT-qPCR in gills and digestive gland according to the exposure conditions and time. Global profile of gene expression responses are clearly different according to the salinity and both organs selected were responding to NP contamination. Results obtained for NPs extracted from commercial product have highlighted the need of further studies using consumer available NPs. The second study was about comparing expression level of the 12 selected genes in both organs after 28 days in control organisms. Interestingly, tissues expression gene analysis revealed strong differences of expression level (>2 fold) for some genes in gills compared to digestive gland regardless to salinity. This work followed expression of 12 genes that showed expression variation according to medium salinity, organ or $nCeO_2$. Studying gene expression changes is an earlier way to detect contamination impact. Genome sequencing of C. fluminea should allow to analyze more gene expression involved in other molecular pathway potentially impacted.

TH003
Adverse effects of BDE-47 on life cycle parameters, antioxidant system, and activation of MAPK signaling pathway in the rotifer Brachionus koreanus
J. Park, J. Lee, Sungkyunkwan University
2,2',4,4'-tetrabromodiphenyl ether (BDE-47) is widely dispersed endocrine disrupting chemicals (EDCs) in the aquatic ecosystem. Due to its devastating effect on marine organisms and insufficient database on toxicology, we investigated the adverse effects of BDE-47 on life parameters and antioxidant defense system following the reactive oxygen species (ROS) production in the monogonont rotifer *Brachionus koreanus*. In *B. koreanus*, the reduction in life cycle, fecundity, and population growth were observed in response to BDE-47. 50 µg/L BDE-47 significantly reduced ($P < 0.05$) life expectancy and net reproductive rate. In response to 10 to 50 µg/L BDE-47 exposure, the oxidative stress was elicited via the generation of ROS, while the antioxidant related enzymes (e.g. glutathione *S*-transferase [GST] and glutathione reductase [GR]) have demonstrated significant activity levels ($P < 0.05$) to further alleviate the oxidative stress in a concentration dependent manner. Furthermore, transcript profiles of antioxidant function (*GST-A*, *-O*, and *-S1-S8*)-related genes have shown the significant increase over 24 h in response to BDE-47 (0, 10, 25, and 50 µg/L). As for MAPK signaling pathway analysis, up-regulation of their activities was observed at 25 µg/L BDE-47 but their activities have reduced at adult NOEC concentration of 50 µg/L. This study provides a better understanding of the effects of BDE-47 on life parameters, molecular defense system, and activation of MAPK signaling pathway against generated oxidants in the rotifer.

TH004
Developing biomarkers of monoamine and neural development in crustaceans.
R. Lock, C. Morris, M. Seifi, J. Swinny, University of Portsmouth; A. Ford, University of Portsmouth / Biological Sciences
Numerous active pharmaceutical ingredients (APIs), which primarily enter the environment due to human excretion into wastewaters, have globally been detected in the aquatic ecosystems. These APIs have been demonstrated to effect various non-target organisms, such as fish and crustaceans. With antidepressants being one of the highest group of prescribed drugs, there is a growing interest into what possible ecological effects they may be having on wildlife. Since entering medical use thirty years ago, Fluoxetine (Flx), a type of selective serotonin reuptake inhibitor (SSRI), is still one of the most popular antidepressants prescribed. Serotonin is one of the chemicals which controls behaviour in all animals. Whilst altering emotional based behaviours in humans, serotonin is involved in multiple biological pathways in invertebrates. Despite the main role of Flx being to concentrate serotonin in synaptic clefts through blocking of serotonin reuptake proteins (SERT), some pharmacologists have described them as 'not so' selective reuptake inhibitors for the ability to bind to multiple neural receptors. SSRI exposure is known to effect monoamine synthesis and neural development. Therefore, this creates a question as to how SSRIs may affect the expression of monoamine and neural function/generation in non-target organisms. This project aims to develop immuno-histochemical techniques to determine monoamine and neural development markers in the crustacean *Echinogammarus marinus* and *Palaemonetes vulgaris*. Preliminary experiments so far have successfully mapped serotonin and dopamine expression across the ventral nerve cord in *E. marinus* indicating that mammalian antibodies show good cross reactivity in these invertebrates. Interestingly, both serotonin and dopamine were localised in their expression and produced and releases throughout the haemocoel. Experiments are ongoing to quantitatively link changes in behaviour to Flx exposure and changes to expression of neurotransmitters and neural biomarkers development in *P. vulgaris*.
Keywords: Fluoxetine, SSRI, Crustacean

TH005
Biomarkers of intersexuality and reproductive dysfunction in crustaceans
S. Short, University of Portsmouth / Biological Sciences; G. Yang, Y.Z. Guler, A. Green Etxabe, University of Portsmouth; P. Kille, Cardiff University; A. Ford, University of Portsmouth / Biological Sciences
Despite the well documented cases of endocrine disruption in vertebrates and some molluscan groups, it is currently unknown whether reproductive endocrine disruption represents a threat to crustacean populations. The study of widespread intersexuality provides an opportunity to investigate sexual differentiation and dysfunction. Crustacean intersexuality is associated with contamination and includes forms linked to increased sex-ratio distorting parasites at polluted sites. Despite the importance of crustaceans for monitoring vulnerable aquatic habitats, little is known about the molecular basis intersexuality or any associated sexual dysfunction. To increase the relevance of crustaceans to environmental toxicologists, this study comprehensively analysed gene expression in amphipods presenting parasite and non-parasite associated intersexuality. We suggest existing vertebrate biomarkers of feminisation should not be applied to crustaceans, as orthologous genes are not induced in feminised amphipods. Furthermore, in contrast to vertebrates, where feminisation and intersexuality is often associated with deleterious de-masculinisation, we find males maintain masculinity even when unambiguously feminised. This reveals a considerable regulatory separation of the gene pathways responsible for male and female characteristics and demonstrates that evidence of feminisation (even if detected with appropriate biomarkers) is not a proxy for de-masculinisation in crustaceans. This study has also produced a comprehensive spectrum of potential molecular biomarkers that are currently being applied to specimens collected from clean and industrially impacted locations. This will represent the first application of appropriate transcriptomic biomarkers to monitor feminisation and de-masculinisation in field populations of crustaceans.

TH006
Development of biomarker genes for endocrine disruption assessment in the non-model crustacean species Gammarus fossarum
D.D. Gouveia, Irstea Lyon / UR MALY   Laboratoire Ecotoxicologie; F. Bonneton, Institut de Génomique Fonctionnelle de Lyon / Génomique du Développement et Évolution.; C. Almunia, CEA; J. Armengaud, CEA / Laboratory Innovative technologies for Detection and Diagnostics; O. Geffard, a. chaumot, Irstea / UR MALY   Laboratoire Ecotoxicologie
Despite the importance of invertebrates in aquatic systems, we lack specific biomarkers to assess the effects of endocrine disruption (ED) on these animals. This can be attributed to a poor knowledge of their endocrine systems and to the absence of genomic data for non-model species. The development of relevant tools for hazard assessment of ED is currently a challenge for crustacean species used in aquatic ecotoxicology. Our research teams have conducted a "proteogenomic" approach to generate a large transcriptomic and proteomic dataset for the amphipod crustacean *Gammarus fossarum*, a sentinel species used widely in aquatic ecotoxicology in Europe. However, among the 2257 MS-certified proteins obtained with this approach, key proteins involved in hormonal regulation that could be potential candidates for endocrine disruption biomarkers were not identified. Therefore, the aim of this study was to identify, in *G. fossarum*, some key players involved in the endocrine regulation of crustaceans/amphipods in order to propose candidate biomarkers of endocrine disruptions in this species. For this, we first established a list of candidate genes known to play an essential role in hormonal systems of crustaceans and insects: nuclear hormone receptors, other regulatory genes, enzymes of the hormone metabolism and hormone-regulated genes. Using sequence similarity and phylogenetic analyses, we identified similar sequences of

three candidate genes in our transcriptomic *G. fossarum* database: the nuclear receptors RXR and E75, and the regulator broad-complex. The three genes were cloned in order to obtain reliable nucleotide sequences and primers for the subsequent expression studies. Their functional validation and relevance as biomarkers was then performed by studying gene expression during the female reproductive cycle, and after laboratory exposure to model ED chemical compounds (pyriproxyfen and tebufenozide).

TH007

Coupled application of antioxidant defence response and embryo development in amphipod crustaceans in the assessment of sediment toxicity

N. Berezina, Russian Academy of Sciences / Zoological Institute; K.K. Lehtonen, A. Ahvo, Finnish Environment Institute / Marine Research Centre

In the Baltic Sea marine environment, chemical pollution and bottom hypoxia are among the main factors responsible for the decline in habitat quality. In the present study, mortality rate, oxidative stress responses and embryo development in the benthic amphipod *Gmelinoides fasciatus* were evaluated with the aim to examine effects of toxic sediments on this species and to verify the possibility to use these endpoints in sediment toxicity evaluation (biotesting), and in marine environmental assessments in general. A highly contaminated sediment sample from the Baltic Sea was diluted with reference sediment from a clean site to come up with series of five test sediments with dilutions from 1:32 to 1:1024. The lowest dilution contained 6064 μg kg dw^{-1} of polyaromatic hydrocarbons, 2030 μg kg dw^{-1} of tributyltin, 2862 μg kg dw^{-1} of total organotins, and various trace metals such as Cu, Pb, Zn and Cd in concentrations of 352, 115 159, and 0.1 mg kg^{-1}, respectively. As a first step, survival rate (%) of *G. fasciatus* was evaluated in a 10 d toxicity test, showing 100% mortality in the 1:32 dilution of the toxic sediment, and no mortality in the reference sediment or in 1:256 and 1:1024 dilutions. Next, after a 28 d exposure, various types of morphological aberrations in embryos were observed in >60% in 1:64 and 1:128 dilutions, significantly more in comparison to the referencetreatment (< 5%). In the 1:256 dilution the total number of aberrations was lower (31%) compared to the two other dilutions but the frequency of malformed embryos remained at an elevated level (>11%) indicating that the embryos were still affected by the levels of contaminants in this treatment. A low number of malformed embryos per female were observed in the reference (< 5%) and the 1:1024 dilution (6%). Elevated activities in glutathione *S*-transferase (GST) and catalase were observed in the contaminated sediments compared to the reference treatment. Significantly higher levels of GST and lipid peroxidation were recorded in the less contaminated 1:1024 sediment compared to the 1:256 treatment, reflecting accelerated function of the cellular detoxification machinery which is able at this level of sediment toxicity to protect cell membranes from extensive damage. The results obtained illustrate the effectiveness of the combined application of molecular biomarkers and effects measured at higher biological levels in sediment toxicity evaluation.

TH008

Developmental/reproductive effects and expression of ecdysone-related genes in Chironomus riparius after exposure to reclaimed water and its fortification with triclosan and carbamazepine.

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Wastewater reuse includes wetland enhancement and restoration. Wetlands naturally provide water conservation by regulating the hydrologic balance and the application of reclaimed water (RW) to wetlands can bring a beneficial use. This potential benefit could turn into adverse environmental impacts when RW doesn't meet the quality criteria to ensure the preservation of wetlands. RW can contain complex mixtures of micropollutants that can be accumulated in sediment affecting benthic fauna. *Chironomus riparius* is a sediment species widely used in Ecotoxicology. There are four OECD standard methods for testing acute, developmental and life-cycle effects on *C. riparius*. Furthermore molecular biomarkers have been successfully applied to explain mechanisms of action of pollutants in *C. riparius* larvae. We therefore designed this study to assess life-cycle effects (emergence, sex ratio, development rate and reproduction) and ecdysone-related gene expression (vitellogenin and ecdysone receptor) of *C. riparius* exposed to RW. The experimental design also included single or binary fortifications of both controls and RW, obtained by adding two emerging pollutants: triclosan (0.02 mg/L) and carbamazepine (0.1 mg/L). Presence of triclosan and carbamazepine in RW together with 21 additional emerging pollutants was analysed. Analytical measures of sediments and overlaying water were also made to know the water-sediment distribution for fortifications. RW consists of a number of recalcitrant organic compounds and this fortification approach should simulate a simple mixture exposure under laboratory conditions that could be useful for result interpretation. Our aim was to elucidate whether *C. riparius* developmental and reproductive effects can be induced by RW and if there are relationships between these apical endpoints and the transcriptional variations of the studied genes. This work has been funded by the Spanish Ministry of Economy, Industry and Competiveness through CTM2012-37547, CTM2013-44986-R and CTM2014-52388-R projects.

TH009

The effects of copper and diuron on the activity of the sodium/potassium and calcium-ATPase during the embryo-larval development of the Pacific oyster, Crassostrea gigas

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The contamination of aquatic environment by pesticides and the associated toxic effects on the marine organisms are widely recognized. Many studies described the impacts of copper and diuron on the early stages of development of the Pacific oyster, *Crassostrea gigas*, but less is known on the underlying toxicity mechanisms. In this study, we investigated the role of a potential disruption of Na^+/K^+-ATPase and Ca^{2+}-ATPase ion pumps in the embryotoxic effects of copper and diuron on oyster larvae. In some fish and invertebrate species, these ion pumps are known to be involved in both the embryo-larval development and homeostasis processes by the regulation of the ion balance. Many studies already demonstrated an inhibition of ion pumps by pollutants such as metals and polycyclic aromatic hydrocarbons. During our study, oyster embryos (1h post-fecondation) were exposed to 4 nominal concentrations of copper (0.10, 1.00, 10.00, and 20.00 $\mu g.L^{-1}$ Cu^{2+}) and diuron (0.01, 0.05, 0.10 and 0.50 $\mu g.L^{-1}$). Embryotoxicity was monitored 24 hpf by determination of the percentage of D-larvae presenting shell and mantle abnormalities. A part of the D-larvae was recovered by sieving in order to measure *in vitro* Na^+/K^+-ATPase and Ca^{2+}-ATPase activity following the adapted protocol from Morgan *et al*. The assay is based on the measurement of inorganic phosphate release by the ATPase enzyme. Our results show a significant embryotoxic effect of copper and diuron respectively from 10 $\mu g.L^{-1}$ and 0,05 $\mu g.L^{-1}$. Preliminary results on ion pumps show a good sensibility and reproductibility of the assay. The measurement of Na^+/K^+-ATPase and Ca^{2+}-ATPase activities in exposed embryos are ongoing.

TH010

Suitabiltiy of Enchytraeids as Indicator Organisms for Nanoparticle Toxicity in Terrestrial and Aquatic Systems

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Soil enchytraeids play significant role in improving the soil pore structure and the degradation of organic matter. They are used as standard organism models for chemical testing, using survival and reproduction as endpoints, and in recent studies biochemical markers. Both titanium-dioxide and zinc-oxide nanoparticles are given considerable attention due to their use in ultraviolet light protection and as catalysts. It is known that both Ti and Zn nanoparticles interact directly with tissue, but they also cause oxidative stress. In this study, *Enchytraeus albidus* was exposed to different concentrations of both nano and bulk forms of titanium-dioxide and zinc-oxide using three different exposure routes. Artificial soil was used for a terrestrial system and soil extract and reconstituted water for aquatic systems. The measured endpoints were survival of organisms and biochemical markers; catalase (CAT), acethylcholinesterase (AchE) and glutathion S-transferase (GST). The results showed that soil extract was not suitable as medium for testing chemicals since it showed a 100% mortality. Both titanium-dioxide and zinc-oxide were toxic in bulk and nano form in artificial soil and reconstituted water, although chemicals in reconstituted water appeared to be more than 1000 times more toxic than in soil.

TH011

Phenotypic defects in Gammarus fossarum exposed to fenoxycarb as embryo

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The use of embryos in ecotoxicology can be useful not only for understanding the mode of action of a toxic compound but also to detect subtle toxic effects as embryos could be the most vulnerable stages within the life history of an organism. Despite these advantages, ecotoxicological studies dealing with embryos of aquatic arthropods remained rare and many use hatching success as the endpoint of interest. In the present study, embryos of the amphipod *Gammarus fossarum* were directly exposed throughout the entire embryo cycle to increasing fenoxycarb concentrations (0, 0.5, 5 and 50 $\mu g.L^{-1}$). Fenoxycarb is a growth regulator insecticide, analog of the arthropod juvenile hormone. In newly hatched individuals from exposed embryos, three types of phenotypic defects were measured: i) eye pigmentation impairment, ii) appendage shape variations by geometric morphometrics and iii) tissue damages. Fenoxycarb at concentration of 5 and 50 $\mu g.L^{-1}$ delayed embryo hatching. Eye pigmentation was impaired in the 5 $\mu g.L^{-1}$ exposed group. Gnathopod centroid size was increased in the 5 $\mu g.L^{-1}$ exposed group while antenna centroid size was reduced in the 50 $\mu g.L^{-1}$ exposed group. Additionally, we detected digestive tract tissue damages in the 50 $\mu g.L^{-1}$ exposed group. This gammarid embryogenesis assay provides a novel and interesting addition to existing assays for testing toxic compounds with gammarids.

TH012

Acute toxicity assessment of sediment in an area under the influence of a chlorinated outfall with Parhyale hawaiensis

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One solution for sewage disposal in several countries is primary treatment followed by chlorination and its discharge in the sea far from the beaches. In Santos city, São Paulo, Brazil, 1 million of cubic meters of urban effluent is discharged, 4.5 km from the beach. Several old and emergent contaminants, their transformation and chlorinated products are expected to be present in those samples. The final destination of those contaminants is the sediment where persistent compounds will accumulate. To assess the toxicity of environmental samples it is important to use species that are representative of the ecosystem we want to protect This study is part of a broad project aimed at the establishment of P. hawaiensis, a circumtropical marine amphipod species as a test organism. This work explored the use of P. hawaiensis in acute toxicity tests of sediment samples collected in an area under the influence of the Santos sewage outfall. We exposed P. hawaiensis neonates to sediment elutriate, pore water and whole sediment samples from 3 sites 40, 20 and 10 meters each (S1, S2 and S3 sites, respectively) far from the sewage outfall. Neonates (< 7 days old) from laboratory culture conditions and placed individually in 96 wells microplates for elutriate and pore water and in 12 wells with 1 g of sediment and 4 mL of reconstituted saline water. Tests were performed for 96h, 24 °C, 12:12 light-dark. No significant mortality of P. hawaiensis neonates exposed to the pore water or elutriate samples was observed. For the whole sediment test, 42, 92 and 50% mortality were verified in samples from S1, S2 and S3 respectively. The sediment of the area seems to be adversely affected by the outfall. More analysis are under way to confirm these findings.The protocol developed for sediment testing with P. hawaiensis neonates seems to be an adequate to monitor the quality of those samples.

TH013

Sediment-associated Cu influence T tubifex: Bioturbation & correlation among individual-level endpoints

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Sediments are known to serve as both source and sink of contaminants, such as Cu. As a result, sediment-dwelling and especially deposit-feeding benthos are at particular risk from Cu exposure. The oligochaete *Tubifex tubifex* is widespread in freshwater sediments of lakes and rivers. It feeds on sediment particles and plays an important role in the ecosystem by serving as prey and due to its bioturbation activity, which affects the pysico-chemical characteristics of the sediment. Therefore, it is ecologically relevant to examine the influence of contaminants on bioturbation by organisms, such as *T tubife*x. The aim of this study was to examine the effects of sediment-associated Cu on *T tubifex* employing both conventional toxicity-testing endpoints (e.g., mortality and growth), and less commonly used endpoints, such as bioturbation and feeding activity. An experimental approach based on simplistic methods was developed to detect effects on bioturbation and feeding. Two experiments (A and B) were conducted, using similar designs: 7 day exposures to clean or Cu-spiked sediment (6 Cu concentrations). Experiment A: examination of Cu bioaccumulation and effects of Cu on worm mortality, feeding rate (egestion) and growth. Experiment B: examination of Cu effect on worm bioturbation (particle diffusion and maximum penetration depth) by adding a tracer, i.e., green microparticles, on top of the sediment and following particle transport. Adverse effects were detected for all assessed endpoints (bioturbation, egestion, growth and survival) with a slight positive effect at the lowest Cu concentrations and adverse effects at higher concentrations indicating hormesis. Our study shows that it is possible to use simple, non-destructive endpoints, such as fecal layer thickness (a measure of feeding rate) and particle transport (as a measure of bioturbation), and that Cu affects *T tubifex* bioturbation.

TH014

A saponin method for controlling entrapment of water flea

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Some water fleas were reported to be difficult to use for ecotoxicity testing because of entrapment phenomenon. Once entrapment occurs, water fleas can move only in the surface film and cannot escape from surface film themselves; finally they will die with prolonged entrapment.Cetyl alcohol was the most used method to prevent the entrapment of water fleas in previous studies. However, we found that cetyl alcohol was harmful for *Daphnia galeata* ecotoxicty testing because of its toxicity and insolubility. Therefore, we tried to use saponin that is natural surfactants extracted from plants to prevent entrapment of water flea. As results, 1 mg/L of saponin was determined as the optimal concentration for preventing entrapment of water fleas and it was applied to heavy metal acute toxicity assay with negligible adverse effects. The saponin method made the *D. galeata* and *Bosmina longirostris* more useful for ecotoxity testing. *This study was funded by the Korea Ministry of Environment (MOE) as "The Chemical Accident Prevention Technology Development Project".*

TH015

Investigation of the sensitivity towards a-cypermethrin is life-stage dependent in Tenebrio molitor

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Pesticides are widely and intensely used all over the world as a tool to ensure good crops. Pesticides are used to avoid diseases and pest such as insects to harm the crops, and thereby insure farmers the highest yield as possible. Most insects undergo metamorphosis during their lifecycle and have four life stages: egg, larvae, pupae and adult. Pesticides are usually tested for the effect on one life-stage and are usually used specific for that one life-stage. Potentially both the target and the non-target insect can be exposed to the pesticide in other life-stages. \n The current study is investigating if there is a life-stage depending sensitivity towards a-cypermethrin in *Tenebrio molitor*. The study is an initial screening together with a similar study of life-stage dependent sensitivity towards a cocktail of a-cypermethrin and propiconazole. The two initial screenings are a foundation for a long-term study of how sub-lethal exposure of larvae and pupae to a-cypermethrin pesticide alter the sensitivity to a-cypermethrin or a cocktail of a-cypermethrin and propiconazole in the adult stage of *Tenebrio molitor*.\n This study where conducted by exposing the larvae, pupae and beetle with a droplet of a-cypermethrin topical, simulating a natural exposure event. The stage-depending sensitivity where observed during 7 days and the effect measures were: alive, paralyzed and dead. After 48 hours the EC_{50} for the beetles were 116 ng/mL and for the larvae it was 448 ng/mL. This indicates that the larvae were the most robust life-stage. After 96 hours the EC_{50} for the beetles were 199 ng/mL, for the pupae 524 ng/mL and for the larvae it was 351 ng/mL. This indicates that the pupae were the most robust life-stage. After 7 days the EC_{50} for the beetles were 190 ng/mL, for the pupae 112 ng/mL and for the larvae it was 420 ng/mL. The EC_{50} values changed during the 7 days due to recovery of some individuals and postponed mortality. The most robust life-stage were the larvae where there were seen highest recovery, survival and overall the highest EC_{50}-values. The most susceptible life-stage were the pupae and beetle where the highest mortality was observed. During the 7 days mostly all the pupae underwent metamorphosis into the beetle but were deform and dead afterwards. From the observed experimental results, it is considered that there is a stage-depending sensitivity towards a-cypermethrin in T. *molitor* and the most robust life-stage is the larvae-stage.

TH016

Optimization of a flow-through system for Daphnia magna chronic toxicity testing following OECD Guideline 211

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Daphnids are playing the role as representative for aquatic invertebrates in the environmental risk assessment for plant protection products, chemicals and pharmaceuticals. To investigate chronic toxicity, semi-static *Daphnia magna* reproduction tests following Test Guideline OECD 211 have to be performed. So far in many cases this test design is also used for degrading substances, despite the fact that over time (2-3 days) the exposure concentration of the parent test compounds is decreasing and the metabolic products are accumulating. To avoid these disadvantages, studies can be performed in flow-through systems. There are different technical designs for flow-through systems with various advantages and disadvantages regarding adsorption of the test item, water exchange rate, suspension of algae as food supply and the number of individual replicates. We are presenting a new flow-through system for *Daphnia magna* which was designed to optimize the exposure of the daphnids to the test item and to provide optimal environmental conditions for the daphnids. The test medium was prepared in an electronically controlled dosing unit and was then distributed via an automated slewing arm into the exposure flasks, providing minimal absorption surfaces. This highly flexible splitting system allows running up to 20 individually treated replicates. The theoretical medium exchange rate of 30 times for each replicate a day allowed to maintain a parent concentration even for compounds with half-lives of approximately 6 hours and assured that degradation products have not accumulated. These high exchange rates required a sophisticated food supply. A dosing system was developed to provide homogeneous algae suspension which ensures sufficient reproduction. The flow-through system may pose additional stress for the daphnids, since interval dosing of test medium is causing turbulences within the test vessels. To preclude that stress effects are given in the new flow-through system, a reproduction test with a hydrolytically stable test substance was carried out. The stability of the test item ensures the same test item exposure in both test designs (semi-static and flow-through). This comparative test allowed studying stress parameters by comparing the effect level concentrations and the ECx values. With this optimized system, fast degrading substances can be tested accurately with little expenditure of work and under optimal exposure conditions for the reproduction of daphnids.

TH017

Effects of aquatic contamination and food limitation on chironomid larval

morphology

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Since the early 1970s, chironomid (Diptera: Chironomidae) larvae collected from polluted freshwater sites have been observed to often show a higher frequency of deformities than those from unpolluted sites. Chironomid larval deformities have consequently been widely investigated in the laboratory, to determine their potential as a chemical exposure endpoint for ecotoxicological studies. However, the causal links between aquatic contaminants and deformities remain uncertain; our recent meta-analysis demonstrated published laboratory results to be highly variable. This meta-analysis also showed that most published data are potentially confounded by mortality, as—although deformities are a sublethal endpoint—chemicals have usually been tested at lethal concentrations. Lethality-inducing experiments exclude “mortality-prone” larvae from analyses, as killed larvae are not analysed for deformities. Published experiments have therefore selected for “mortality-resistant” larvae, rather than assessing deformity effects in the whole population. Additionally, our knowledge of the factors causal to deformities is limited by a bias towards chemical assays in the literature. Non-chemical stressors are rarely considered, though it is possible that non-chemical factors are inducing the deformities observed in field studies. Our study therefore aims to assess chemical and nutritional stress as deformity-inducing agents in the Australian model chironomid *Chironomus tepperi.* In this laboratory study, we test the association between insecticide (imidacloprid and permethrin) and metal (lead and copper) exposure and deformities. In addition, we also investigate food limitation as a deformity-inducing agent. All stress conditions were sublethal, avoiding the confounding effects of mortality; which have complicated the interpretation of previously published work in the literature. By testing effects at sublethal stress levels, and testing a non-chemical stressor, we clarify the causal links between environmental stress and chironomid deformities; which have remained enigmatic for over 40 years. In clarifying these links, we evaluate the utility of chironomid deformities as an ecotoxicological endpoint.

TH018

Cost effective approaches to assessing the impacts of radiation on aquatic invertebrates: fecundity and fluctuating asymmetry in Asellus aquaticus at Chernobyl

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The effects of long-term, environmentally relevant doses of radiation on biota remain unclear owing to a lack of studies following chronic exposure in contaminated environments. Such studies are necessary to elucidate and mitigate the impacts of both planned and accidental releases of anthropogenic radioactivity on the environment. This study aimed to assess the impacts of thirty generations of radiation exposure on the development and reproduction of the model isopod crustacean, *Asellus aquaticus*, using fluctuating asymmetry and measures of fecundity respectively. Fluctuating asymmetry is defined as random deviations from the expected perfect bilateral symmetry of an organism and has gained prominence as an indicator of developmental stability in both ecotoxicological and ecological studies. *Asellus aquaticus* is a benthic detrivorous isopod commonly used in ecotoxicity testing of sediment-borne contaminants. Organisms were collected from littoral zones of six lakes along a gradient of radionuclide contamination with total dose rates ranging from 0.06 – 27.1 μGy/hr. Nutrient levels and hydrochemical parameters were assessed at each locality. Fluctuating asymmetry was assessed in four meristic and one metrical trait, and brood sizes/reproductive investment measured in gravid females. Significant differences in levels of pooled asymmetry were recorded between sample sites independent of sex and specific trait measured. Observed differences in asymmetry were not correlated with radiation doses (R-squared = 0.0084, $p = .452$) or any of the measured hydrochemical parameters, suggesting that differences in asymmetry were not attributed to radionuclide contamination and were driven by a biotic factor at a single site. No significant differences in brood sizes or reproductive investment were observed amongst sites of varying radionuclide contamination. A significantly greater number of reproducing females at contaminated sites was observed and was not related to any of the measured environmental parameters, suggesting delayed reproduction in contaminated sites. The efficacy of fluctuating asymmetry and fecundity as cost effective tools for monitoring populations chronically exposed to pollutants are highlighted. These findings enhance our understanding of the response of biota to chronic pollutant exposure and will help elucidate long term effects of large scale nuclear incidents such as Chernobyl and Fukushima.

TH019

Behavioral and regeneration endpoints to evaluate impacts of PAHs on planarians

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Every missing part of a planarian can be regenerated, including the nervous system, which shares many similarities to the vertebrate nervous system. This planarian feature can be used to evaluate effects of toxicants, in combination with other endpoints such as locomotion, reproduction or biochemical parameters. Therefore, planarians have the potential to provide new insights on the ecotoxicity of some ubiquitous freshwater contaminants, such as polycyclic aromatic hydrocarbons (PAHs), which continue to be major contaminants resulting from industrial processes. The sexual planarian *Dugesia tigrina,* a common freshwater predator, was used to study the effects of 3 of the most common PAHs, i.e., phenanthrene, pyrene and benzo[a]pyrene. Acute tests were performed over a 96-h period, while sub-lethal endpoints, such as, locomotion, feeding and regeneration were measured over 8-day exposures. PAH concentrations were measured in experimental solutions and organisms by fluorescence spectrophotometry. Interestingly, it was only possible to determine the LC_{50} for phenanthrene (830.1 μg L^{-1}), since no mortality was observed over 96 hours for pyrene and benzo[a]pyrene, despite the high concentrations tested (> 2000 μg L^{-1}). Nevertheless, for the 8-day exposure both pyrene and benzo[a]pyrene impaired feeding, locomotion and regeneration of planarians exposed to lower concentrations than those used for phenanthrene. On the other hand, effects caused by phenanthrene were most pronounced on the feeding activity with a LOEC of 150 μg L^{-1}, approximately 18% of the LC_{50}. These results indicate that the sensitivity of planarians to these compounds may be comparable or lower to the sensitivity of other invertebrates, such as *Daphnia*,at concentrations that can be found near contaminated industrial sites. This study provides relevant data on the toxicity of single PAHs to invertebrates and emphasizes the importance of testing behavioral endpoints, as well as, alternative endpoints, such as planarian regeneration, in order to improve our knowledge concerning the possible impacts of xenobiotics.

TH020

The survival and feeding rate of Daphnia for acute toxicity assessment of water environment

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The efficiency and sensitivity are important parameters of the methods used to determine the water quality using living organisms. Therefore we have investigated the toxic effects of pollutants to *Daphnia magna* that is most commonly used test-organism in bioassay. This toxicity assessment was carried out by two test-functions. The evaluation of *Daphnia* survival in the acute toxicological experiment was carried out according to bioassay method which was previously elaborated by us. This method is more rapid and has ability to control the conditions automatically during the analysis. This opportunity came due to the climate chamber and the device for Crustaceans exposure which were specially designed for. The patterns obtained during the research allowed us to reduce the time for Daphnia survival bioassay from 96 to 48 hours, while keeping a high sensitivity to toxicants. Daphnia’s feeding rate ?hanging can be the other characteristic of the toxic effect. In this case the test-organism’s reaction to the toxicant is registered on sublethal level and consequently is detected speedily. The food (alga suspension) consumption measurements carried out on "Foton-10" fluorometer: the method based on cells fluorescence intensity registration. The high sensitivity of the device allowed working with a small amount of input feed, which is one of the main conditions to reduce the measurement’s duration of the Crustacean’s trophic activity. In experiments with model toxicants (heavy metals) conditions (stocking density, feeding schedule, Daphnia's age etc.) in which the crustaceans demonstrate sufficiently high trophic activity in control variant and sensitivity in the experiment with polluting substances were determined. The higher sensitivity of the feeding rate of Daphnia compared to the index of their survival rate to model toxicants (ions of cadmium, copper and potassium dichromate), and also while carrying out bioassay of sewage and natural waters is shown. In which connection in most cases the toxicity of samples was identified more quickly due to the feeding rate reduction, than by the crustacean’s death.

TH021

Development of a freshwater molluscan embryo bioassay with Lymnaea stagnalis: a beneficial standalone assay, and complimentary appendment to an existing OECD Test Guideline.

C. Stenton, J. Elphinstone-Davis, Cefas

Embryo bioassays are an efficient and cost effective tool for environmental risk assessment. Early-life stages exhibit high sensitivity to chemicals, and provide insight into developmental toxicity. Molluscs have demonstrated high sensitivity to chronic chemical exposure, and although embryo bioassays have been developed for several marine molluscs e.g. the ICES Oyster Embryo-Larval Bioassay, there is presently no recognised equivalent for freshwater species. *Lymnaea stagnalis* is an ideal candidate species for such an assay given their widespread global distribution, ease of culture, and established genomic sequence. This choice is also supported by the recent adoption of OECD Test Guideline 243: *Lymnaea stagnalis* Reproduction Test. The proposed method could either be used as a standalone bioassay, or as a complimentary appendment to the aforementioned test guideline. A 42d static-renewal study was conducted from 16th February to 30th March 2015 on the

RENILYS® strain of *L. stagnalis* provided by the Institut National de la Recherché Agronomique (INRA; France). The study followed the then draft OECD test guideline conditions (photoperiod 16L:8D, 400-500 lux, test vessel temperature 20 ± 1 °C). Mature *L. stagnalis* (27 ± 2 mm) were exposed to cadmium at time-weighted mean concentrations of: 0.0, 5.9, 8.84, 14.33, 21.64, and 31.76 µg/L, and growth and reproduction monitored. The embryo bioassay was sequentially conducted on progeny taken from all concentrations. Three scenarios were tested: embryos produced from control adults transferred to test media; embryos from exposed adults transferred to dilution water only; and embryos taken from exposed adults maintained in test media. Embryo hatching proved a more sensitive endpoint than adult fecundity or embryo survival. In all three scenarios a concentration response was detected for hatching success and time to hatch: a LOEC of 18.44 µg/L was established for both groups of embryos maintained in test media; for embryos transferred to dilution water the LOEC was 31.76 µg/L. Broader investigation of contaminants and developmental endpoints would be prudent, however, low mortality, ability to finely monitor development, and high standardisation support the methodology and demonstrate potential for the implementation of the embryo bioassay for toxicity assessment.

TH022

A comparative assessment of chronic toxicity between freshwater (Daphnia magna) and marine (Tisbe battaligai) test species

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The marine environment can be split into three distinct zones: estuarine, coastal and open ocean. The physio-chemical interactions within these zones and the stresses on the organisms' present are quite different. Estuarine zones for example are highly dynamic with tidal cycles, river flows, rainfall and run off affecting salinity, temperature, turbidity and flow rates whereas open ocean environments have more constant physio-chemical properties due to their volume, depth and circulation. The marine environment has many different test guidelines but none that are comparable to the OECD test guidelines for freshwater organisms. Given the changeable environmental conditions across the different marine environments, do the current test guidelines ensure adequate or comprehensive protection? Another question is whether read across from freshwater toxicity studies is acceptable as an alternative. This project assesses the similarities and differences of test design and developmental/reproductive toxicity of a reference toxicant to the freshwater test species *Daphnia magna* and a marine test species *Tisbe battaligai*. The *Daphnia magna* chronic reproduction study follows the OECD 211 test guideline, three endpoints; survival, growth and reproduction are analysed. This test will be compared to a 21 day chronic *Tisbe battaligai* development and reproduction study which has been used as an alternative to the *Daphnia* magna study for marine risk assessments.

TH023

Validation of a short-term, in vivo screening method for detecting chemicals with juvenile hormone activity using adult Daphnia magna(JHSST)

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Daphnids are a group of Crustacea that are found worldwide and are sensitive to various chemical toxicities. Therefore, Daphnia magna has been used to evaluate the toxic effects of general chemicals. D. magna usually produces only female neonates via parthenogenesis, which have the same genome as their mother. The laying eggs to the brood chamber, development and release are usually completed within 48 h. Male offspring are produced when the environmental conditions worsen, such as low temperature, short photoperiod, lack of nutrition and overcrowding of the biomass (Estrada and Mulla, 1986; Hebert, 1987; Hobaek and Lasson, 1990; Kleiven et al., 1992; Stross et al., 1965). Juvenile hormone (JH) and JH agonists have been shown to induce male offspring production in various daphnids, including Daphnia magna using OECD TG211. The critical period (about 1h) for JH action on ova in the parent's ovary to induce male offspring is existing at 7-8h later from ovulation. Therefore, we considered that adult D. magna could be used to produce a short-term screening method for detecting JH analogs. Using this method, we successfully demonstrated male offspring induction in the second broods after exposure to JH or JH agonists. After investigating the exposure time, the number of repetitions and the exposure concentration, we established a short-term, in vivo screening method for detecting JH analogs using adult D.magna. We examined positive and negative control chemicals using a previously developed method and verified the validity of our new testing method(JHSST;Juvenile Hormone Short-term Screening Test). As a method of the new screening of the juvenile hormone-like substance based on our results of research, Japanese Ministry of the Environment suggested JHSST to OECD. For a validity evaluation of JHSST, a ring test is held by participation more than three countries by the end of this year. We hope for the participation in ring test from many countries.

TH024

Toxicity of microbial insecticides towards the non-target species Chironomus riparius

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The increasing public concern over the continued use of chemicals in the agriculture resulted in the development of new types of pesticides. Microbial insecticides based on live fungi or bacteria and on their toxins have been used as a promising alternative to chemical insecticides in integrated pest management. Despite being considered environmentally safe due to their high selectivity, short half-life and biodegradability, a deeper environmental risk assessment is mandatory. Such approach should be focused on relevant and frequently overlooked ecological effects of these compounds. Particular attention should be devoted to the toxicity of these insecticides on non-target species, including aquatic species. To evaluate the potential ecological effects of microbial insecticides in aquatic ecosystems we examined the life history responses of *Chironomus riparius*, an aquatic non-target species. *C. riparius* larvae were exposed to two commercial microbial insecticides based on the bacterial pathogen *Bacillus thurigiensis* var. *israelensis* (VectoBac® 12AS - VB) and on the fungal pathogen *Beauveria bassiana* (Naturalis®-L - NL). Organismal endpoints such as survival, larval growth and emergence were used. Effects on the energy reserves were also measured. Acute tests presented a 48 h LC_{50} of 1.85 µg/L for VB and of 34.7 mg/L for NL. Regarding sub-lethal effects, different modes of action of these microbial insecticides were reflected in distinct effects on *C. riparius*. VB decreased larvae survival with consequent implications to the percentage of emerged adults in all concentrations while NL reduced development rate (reduced larval growth and delayed emergence) with increasing concentrations but had no effect on the percentage of emerged adults. Moreover, protein content was significantly reduced following a short exposure to both VB and NL, but no effects were observed for carbohydrate or lipid contents. The present study concludes that exposure to these microbial insecticides impaired *C. riparius* survival, developmental rates and emergence, revealing their toxicity towards non-target organisms and potential population level effects. These results add important information for the risk assessment of the microbial insecticides in aquatic ecosystems.

TH025

Infuence of biochar particle sizes on biota responses

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There is an increasing interest for the employment of biochar as a soil amendment and a carbon sink. A robust hazard assessment is necessary to ensure sustainability before large scale implementation can be considered in policy development. This study hypothesized that biota responses depend on biochar particle size distribution and application rates. Pine woodchip biochar was incorporated in soil at two concentrations (1% and 6%) and three particle size classes (≤0.5 mm, 1-2 mm, ≤4 mm), giving 6 treatments. Soil without biochar was used as a negative control. As a first screening, the earthworm avoidance behavior was assessed in the species *E. andrei* in laboratory bioassays. Aiming for an ecologically representative approach, a 28-days mesocosm experiment was conducted looking into survival of *E. andrei* and micro-organisms' feeding activity using the bait lamina method. After 28 days, soil leachates from the mesocosms were collected to assess effects on *D. magna* immobilization and *V. fischeri* bioluminescence. A laboratory feeding experiment with earthworms was also performed and changes in body mass recorded. Overall, the 6% ≤0.5 mm treatment had a statistically significant negative effect on avoidance behavior, on fauna feeding in the mesocosms, and on the body mass of *E. andrei*. Aquatic bioassays resulted in the absence of toxicity to *D. magna* and *V. fischeri.* Further experiments are planned in order to look into mechanisms of biochar effects on biota. Results suggest an urge for inclusion of bioassays in biochar quality assessment, alongside the physicochemical characterization methods proposed within regulatory guidelines. Key words: biochar particles, biota, ecotoxicity

TH026

The enantioselective synergism of the fungicide imazalil with the pyrethroid α-cypermethrin towards Chironomus riparius

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Around 30 percent of the organic chemical pesticides on the market are chiral molecules and are often applied as racemic mixtures, i.e. as a 1:1 mixture of enantiomers. Even though the physicochemical properties of enantiomers are the same, apart from the rotation of polarized light, their biological activity often varies since most enzymes are stereoselective and interact preferably with one of the enantiomers. This can not only lead to differing pesticidal efficacy of the individual enantiomers, but also to differing biotransformation rates and toxicity in organisms. In this study, the relevance of enantioselectivity of chiral pesticides in ecotoxicology and mixture toxicity was explored. Azole pesticides such as imazalil have been suggested to inhibit certain cytochrome P450 enzymes which are relevant in xenobiotic metabolism. Together with pyrethroid insecticides, azole fungicides (triazoles and imidazoles) have been shown to interact synergistically in aquatic non-target organisms (Kretschmann et al. 2015). Different synergistic potencies seen for different azoles was suggested to be partly due to inactive enantiomers in racemic mixtures (Dalhoff et al. 2016). This study aimed to investigate the enantioselectivity in inhibition of cytochrome P450 and in the synergistic potential of the azole fungicide imazalil in the mosquito larvae *Chironomus riparius*. The hypothesis was that the cytochrome P450 inhibition and therefore also the synergy with pyrethroids would vary significantly between the two imazalil enantiomers. Both *in vivo* and *in vitro* studies applying the ECOD assay were performed in order to investigate the enantioselectivity of the cytochrome P450 inhibition. In addition, possible enantiomer - enantiomer interactions were studied. Whether the difference in cytochrome P450 inhibition leads to differing synergy was assessed with the pyrethroid insecticide α-cypermethrin. Altogether, this study wants to highlight the importance of considering enantiomers of chiral pesticides as individual compounds in ecotoxicological tests.

TH027
Comparative toxicity assessment of lead and mancozeb on two Daphnia species.
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Anthropogenic activities have been contaminating natural ecosystems. This contamination can have several diffuse and point sources, from industrial, domestic or agricultural outputs, leading to organisms' exposure to different chemicals. Different species present different sensitivities to those exposures, even for species phylogenetically close related. The goals of this study were to compare the sensitivity of two *Daphnia* species (*D. magna* and *D. similis*) to lead (Pb) and the agricultural fungicide Mancozeb, as relevant chemical models for groups such as metals and pesticides that may occur in the environment. To accomplish that, standard toxicity tests were conducted in order to determine their inherent lethal (immobilization) and sublethal effects (reproduction, feeding inhibition and oxygen consumption). Regarding Pb, the lethal concentration ($LC_{50\text{-}48h}$) for *D. magna* (0.43mg/L) and *D. similis* (0.36mg/L) were similar. A similar pattern was observed for mancozeb with *D. magna* (0.18mg/L) showing similar sensitivity to *D. similis* (0.25mg/L). The reproduction assay was performed and the 50% effect concentration (EC_{50}) derived. The window between sublethal and lethal effects for Pb exposure in *D. similis* was so narrow that no EC50 could be derived; but, for *D. magna* an EC_{50} for Pb could be estimated as 1mg/L. On the other hand, *D. similis* presented a decrease on somatic growth when exposed to Pb, while *D. magna* was not affected. Mancozeb exposure induced effects on reproduction and growth in both species. Reproduction and growth presented a positive correlation in *D. magna* exposed to Pb (Pearson correlation; r=0.84; p< 0.0001) while for *D. similis* size and reproduction were not correlated (r=0.07, p=0.58). Regarding mancozeb exposure, reproduction and growth were not correlated for both species. Results show that D. magna is more tolerant than D. similis when exposed to Pb. Relative to Mancozeb, sublethal and lethal effects have similar results regarding both species. Data suggest that these contaminants affect both species in different ways.

TH028
Functional endpoints in ecotoxicology: a case study on the benthic community in freshwater indoor microcosms
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Higher tier studies in ecotoxicology such as aquatic microcosm experiments typically focus on biodiversity and species abundance. These endpoints provide useful information of toxic effects on the structure of populations and communities. However, they fail to include potential effects on the function such as secondary production. The present 7-month microcosm study investigated effects on biomass (B), secondary production (P), and their relationship (biomass turnover ratios, P/B) in comparison to standard parameters of abundance and diversity on almost the entire benthic community. Cadmium in low and high concentrations (nominal 50 and 400 mg Cd kg^{-1} dry sediment) served as a model toxicant to enable a relative constant disturbance such that the underlying principles of chronic stress on populations and communities could be studied. Results show that B, P and P/B have different sensitivities compared to abundance and diversity with acceptably low variance in the data. Specifically, P/B of whole benthic proto- and metazoan communities increased under Cd stress, probably due to a shift within the communities to a higher proportion of fast reproducers (r-strategists). Likewise within the metazoans, Cd stress tends to provide favorable conditions for taxa with relatively short development times and high turnover rates (e.g. ostracods and rotifers) than for taxa with longer development times and lower turnover rates (e.g. oligochaetes and copepods). This effect was also found for nematodes on a species level. Further, secondary production of nematodes appeared to be relatively independent of species diversity and, concurrently, production was more sensitive than species richness. The study therefore provides novel insights into the structure and function of the benthic community under toxic stress.

TH029
The impact of pesticides on the quantitative composition of the cellular components (coelomocytes) of the hemolymph with coelomic fluid of earthworms (Eisenia fetida)
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Earthworms are well known soil poisoning identifiers and a number of standards, guidelines and recommendations related to ecotoxicological studies on earthworms were issued. Methods for assessing the toxicity of chemicals described in these documents are based on four indicators - mortality, reproduction, body weight and behaviour. However, scientists are still looking for new indicators that will allow for more accurate estimate of the toxicity of chemical substances. The aim of this study was to investigate whether exposing earthworms to different kinds of pesticides affects the quantitative composition of cellular components of the hemolymph with coelomic fluid (coelomytes). The experiment was performed on the adult earthworm, *Eisenia fetida* (Savigny 1826) obtained from a culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna. All worms have reached maturity and their clitella were developed. The earthworms were exposed to 8 different pesticides (insecticides, fungicides and herbicides) for 4 weeks during the experiment. Simultaneously untreated control groups were conducted. Ten worms from treated (the highest concentration of the test material which does not kill at least half of earthworms) and control groups. After completion of experiments 10 worms from each test item and controls underwent three days of starvation in order to clean the digestive system. Next, after a few minutes of cooling, an earthworm was delicately incised at the height of 5-6 last segments, and the liquid that appeared was immediately smeared on a microscope glass slide. The collected smears were stained with Hemacolor® Rapid staining of blood smear. Approximately 50 cells per sample were analyzed. During the microscopic evaluation, such cells as basophilic and acidophilic leucocytes, eleocytes, granulocytes and neutrophils were counted. Statistical analysis of the results showed statistically significant decreases and increases of the number of cells in all cell types depending on the test item. Two test items deserve special attention: carbendazim and chloroacetamide (reference item and positive control in toxicity tests on earthworms) which caused the decrease in the number of all observed cell types. It can be concluded that the evaluation of smears the hemolymph with coelomic fluid may be an additional endpoint in assessing the effects of chemicals on soil organisms, but further studies should be conducted.

TH030
The comparison of the sensitivity of the collembolan, Folsomia candida and the earthworm, Eisenia fetida to pesticides
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Pesticides play an essential role in maintaining high levels of food production, but on the other hand, they affect soil fauna and many biological processes involving it. The aims of the presented study were (i) to compare the sensitivity of test organisms to selected insecticides, herbicides, and fungicides, and (ii) to assess the impact of pesticides belonging to different groups on reproduction of the earthworm, *Eisenia fetida* and the collembolan, *Folsomia candida*. Both earthworms and collembolans are integral parts of the soil ecosystem and are sensitive to soil contaminants. The experiments were performed according to OECD Guidelines No. 222 and 232. Three insecticides (acetamiprid, indoxacarb, and alphacypermethrin), three herbicides (metamitron, mesotrione, and s-metolachlor), and three fungicides (dodine, dithianon, and thiophanate methyl) commonly used in agriculture were chosen to be used in them. The tested pesticides contained different active substances. They also varied in terms of their content. Aqueous suspensions/solutions/emulsions of the pesticides were applied to the soil. There were also concurrent control groups. On the basis of the results, the EC_{10}, EC_{20}, EC_{50}, and NOEC were calculated using the ToxRat Professional statistical computer software. Available literature data indicate that collembolans are strongly sensitive to insecticides due to their close phylogenetic relationship to insects. In contrast to this, the obtained results showed various impact on the collembolan and the earthworm reproduction, which did not depend on the type of the pesticide used in the study. It was noticed that the collembolans were more sensitive to the pesticides containing acetamiprid, metamitron, and s-metolachlor than the earthworms. No significant impact of metamitron on the *Eisenia fetida* reproduction was observed. The pesticides which include indoxacarb, alphacypermethrin, mesotrione, dodine, dithianon, and thiophanate methyl strongly impacted the earthworms. There was no significant effect of alphacypermethrin and dithianon on *Folsomia candida* reproduction. Therefore, it would seem justified to perform ecotoxicology studies on various representatives of soil fauna. The data may indicate that the impact on the reproduction of the tested species depends on functional groups of the active substance molecule, particle size, and metabolites.

TH031
Combining assessment of apical endpoints and gene expression on the freshwater snail Physa acuta after exposure to reclaimed water.
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Fluidos; P. Sanchez-Arguello, INIA - National Institute for Agricultural and Food Research and Technology / Environment
OECD has recently adopted two guidelines for testing reproductive effects in freshwater snails highlighting the need for testing toxicity in this group of invertebrates. Previous to test standardization many studies have proven the suitability and sensitivity of gastropods for detecting reproductive effects induced by pollutants, including endocrine disrupting compounds. In addition to endpoints of new OECD guidelines, embryotoxicity/egg quality of gastropods has been used for testing teratogenicity or even for detecting alterations of reproductive pathways by complementary assessment of offspring embryonic development in a reproduction test. Nevertheless there is a lack of knowledge in Gastropoda endocrinology because proteomic and genomic methods are scarcely applied in gastropods. Thereby relationships between mechanisms of action and reproduction effects in gastropods are still poorly understood. Molecular biomarkers are able to detect pollutants by their mechanism of action and this is especially useful for evaluating complex mixtures like reclaimed water (RW). In fact it has been recommended the use of *in vitro* bioassays/bioanalytical methods as complementary quality assessment together with traditional physico-chemical analysis for wastewater reuse. Nevertheless there are many uncertainties linking molecular effects to whole organism responses. Our aim was to compare early and long-term responses produced by whole and diluted RW. This study combines the assessment of effects at molecular level (gene expression of ERR, RxR and Hsp70) and apical endpoints (fecundity and hatching of offspring) in the freshwater snail *Physa acuta* after exposure to RW. Since RW can contain a cocktail of micropollutants this approach includes a laboratory mixture used for fortification of control and RW by adding three compounds (methylparaben, PFOS and fluoxetine). Adequacy of methods for ecotoxicity assessment of RW, differences in sensitivity and correlations between gene expression and apical endpoints are discussed herein. This work has been funded by the Spanish Ministry of Economy, Industry and Competiveness through CTM2013-44986-R project. M. Aquilino is the receiver of a predoctoral contract Ministry of Economy, Industry and Competiveness (BES-2013-064041).

TH032

Development and test of a new micro-impedance sensor system for water quality monitoring

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Continuous biological monitoring of the toxic potential generated by pollutant cocktails in surface waters has already been established, e.g. biomonitoring of large transboundary rivers in Europe. 95% of our freshwater resources are subterraneous groundwater, which often serves as drinking water. However, groundwater ecosystems and their responses to pollutants still need to be investigated. Pollutant concentrations in groundwater habitats are much lower than in surface water bodies, therefore, we need more sensitive bioindicator species for online biomonitor systems. Within a bilateral ZIM cooperation project we jointly developed a new and highly sensitive micro-impedance sensor system (MSS) which is able to record the behaviour of individuals of 1-2 mm size, thus allows to use representatives of meiofauna, which are the dominant fauna in groundwater biocoenoses. The MSS has successfully been tested and calibrated with *Eucyclops serrulatus* (Copepoda). Compared to macrocrustaceans from freshwater and groundwater habitats, *E. serrulatus* proved to be more sensitive towards chronic exposure to BPA. Easy culture and handling additionally opt for *E. serrulatus* as new indicator in biomonitors.

TH033

Ecotoxicological assessment of phosphate recyclates from sewage sludges

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Sewage sludge contains valuable plant nutrients, especially phosphorus. But even heavy metals and organic pollutants like pharmaceuticals, pesticides etc. are components of the so-called biosolids, therefore new technologies for phosphorus recovery from sewage sludge are necessary. For recycling of phosphorus as a fertiliser in agriculture in form of crystallised (struvite) or thermally treated P-recyclates (PRs), the toxic potential on target species of affected environmental compartments (soil, water, sediment) has to be tested. The direct acute toxic effects of the PRs and a conventional phosphate fertiliser (TSP) were assessed with 1) the freshwater shrimp *Gammarus fossarum* by mortality and behaviour (decrease of movement activity and feeding behaviour) 2) the duckweed *Lemna minor* by growth inhibition, decrease of biomass and growth length 3) the tiger worm *Eisenia fetida* by its avoidance behaviour. Additionally, the eluates of the two most effective products in the direct assessment of ecotoxicity were tested by *G. fossarum*, the most sensitive species in this study. TSP was more toxic than the PRs at the higher tested concentrations (5-10 g DM l^{-1}, 5-50 g DM kg^{-1}), probably due to a higher water solubility and not to chemical composition. Higher concentrations of the crystallised PRs (5-10 g DM l^{-1}) caused mostly a slightly higher negative effect on tested parameters of the duckweed and the freshwater amphipod than the thermally treated PRs. Agronomical relevant application amounts of all PRs and TSP (worst-case scenario) might not have an acute toxic effect on the soil invertebrates.The PRs might have minor effects on the growth of *L. minor* and TSP might negatively affect the survival of the freshwater amphipods. Re-using PRs, in particular struvite with low pollutant concentrations, could be one of the future alternatives of phosphorus fertilisation in agriculture. The research project P-REX is supported by the European Commission under the Seventh Framework Programme (Priority: "From Prototype to Market"/ Contract No. 308645).

Fish model species in human and environmental toxicology (P)

TH034

Comparison of different Fish Toxicity Test Guidelines: Scientific Output vs. Investment and Costs

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The OECD provides different test guidelines for evaluating the toxicity of chemicals to fish. The guidelines cover different test designs from acute and short term to chronic toxicity tests with different durations and endpoints. The Eurofins Agroscience Services Ecotox laboratories have considerable experience and compared the output of the different test designs based on studies with the zebrafish *Danio rerio* as reference species. Acute toxicity tests are described in OECD guidelines 203 (Fish Acute Toxicity Test) and 236 (Fish Embryo Acute Toxicity Test) both with an exposure time of 96 hours. A short-term toxicity test with a duration between 8 and 10 days is recommended by OECD guideline212 (Fish Short-term Toxicity Test on Embryo and Sac-fry Stages). Guidance for chronic toxicity tests is delivered in OECD guidelines 215 (Fish Juvenile Growth Test) and 210 (Fish Early Life-Stage Test) with experimental phases of 4 and 5 weeks, respectively. Due to the different test durations, costs differ drastically and because different life-stages are covered, various endpoints can be evaluated. From this relationship, an output-to-cost ratio was estimated for all tests. OECD guidelines 212 and 215 ended up with the poorest ratios. A medium ratio was derived for OECD guidelines 203. The best output was provided by OECD 236. Although it is the most cost-intensive test, OECD 210 also has an excellent ratio due to the large number of endpoints evaluated during different life-stages. From our laboratory experience, we are unable to recommend OECD 212 and 215 due to their very low output to costs ratio. Furthermore, in OECD 215, the use of juvenile fish is recommended, which is questionable with respect to animal welfare. Juvenile fish are exposed in OECD 203 as well, but the test is cheap due to its short duration. Moreover, its output is of high informative value since historical data is available for a lot of chemicals. The acute test described in OECD 236 has the potential to replace OECD 203, but it is not always suitable and therefore not accepted by regulators, although LC-values evaluated with OECD 236 are often comparable to those of OECD 203 and the use of fish eggs/embryos poses reduced concern regarding animal welfare. OECD 210 is expensive because of its long duration, but it has the enormous advantage that the most sensitive life-stages of fish are covered. To our opinion, this is the most valuable test to evaluate potential risks of chemicals to fish.

TH035

Developmental toxicity of cyanobacterial water bloom extracts

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Cyanobacteria produce and release to the aquatic environment various types of bioactive compounds, which could cause adverse effects in organisms. They are most significantly released into surface waters during cyanobacterial blooms, but there is only scarce information on their potential relevance for effects, especially *in vivo*. Numerous hints of presence of unknown bioactive compounds were reported pointing for example to retinoid-like compounds. The aim of this study was to determine bioactive and teratogenic potential of natural complex mixtures of compounds produced by cyanobacterial water bloom field samples. In this study bioactivity was characterized by means of *in vivo* and *in vitro* bioassays and chemical analysis of active compounds. Special focus was given to samples with bioactivity that could not be explained by presence of known toxins such as microcystins. Samples were taken from water bodies in the Czech Republic, processed, concentrated, and tested using the *in vitro* bioassays for determining the total retinoid-like activity. Teratogenic potency was assessed by analysis of developmental disorders and monitoring of early zebrafish development. The samples were also fractionated and analyzed by chemical analysis to characterize compounds contributing to the effects observed in bioassays. *In vitro* bioassays showed significant total retinoid-like activity in collected field samples.

Retinoid-like activity corresponded to the results of *in vivo* fish embryo tests, where interferences with development were detected. Malformations typical for retinoid signaling disruption were detected after exposure to the extract of cyanobacterial biomass and those were compared to effects of standard retinoid compounds. The effects showed high morphological resemblance and the same dose-response pattern, suggesting presence of compounds affecting retinoid signaling in early vertebrate development. Based on the results of bioassays chemical analysis of retinoid compounds was conducted in active fractions of samples. Those compounds were detected in ng - mg/L range in selected samples and correspond with effects detected by bioassays. However, measured concentrations do not completely explain the toxic effects of mixture suggesting there are more compounds with retinoid-like activity contributing to the toxicity. This work was supported by the Czech Ministry of Education, Youth and Sports (LO1214 and LM2015051).

TH036
Carbon nanopowder acts as a Trojan-horse for benzo(a)pyrene in Danio rerio embryos
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Carbon-based nanoparticles are largely distributed worldwide due to fossil fuel combustion and their presence in many consumer products. In addition to their proven toxicological effects in several biological models, attention in recent years has focused on the role played by carbon-based nanoparticles as Trojan-horse carriers for adsorbed environmental pollutants, such as pesticides, herbicides, benzene derivatives, pharmaceutical compounds and hydrocarbons. This role has not been conclusively determined to date because carbon-based nanoparticles can decrease the bioavailability of contaminants or represent an additional source of intake. Herein, we evaluated the intake, transport and distribution of a carbon nanopowder and benzo(a)pyrene, when administered alone and in co-exposure to *Danio rerio* embryos. One of the crucial points of this study was the choice of an experimental design different from the classical plan, in which the physical and chemical compounds are supplied contemporarily but separately to the biological model. We created a carbon nanopowder doped with a specific quantity of benzo(a)pyrene through a preliminary clean-up of the carbon nanopowder, eliminating the previously adsorbed PAHs, followed by doping of the clean carbon nanopowder with benzo(a)pyrene. This foresight allowed us to assess the fate and effects of benzo(a)pyrene exclusively adsorbed on the carbon nanopowder without any interference due to the hydrocarbon dissolved in water. Data obtained by means of advanced microscopic techniques illustrated that the "particle-specific" effect induced a modification in the accumulation of benzo(a)pyrene, which is forced to follow the distribution of the physical pollutant instead of its natural bioaccumulation. The combined results from biomarkers, functional proteomics and gene transcription analysis highlighted the different biochemical pathways involved in the action of the two different contaminants administered alone and when bound together. In particular, we observed a clear change in several proteins involved in the homeostatic response to hypoxia only after exposure to the carbon nanopowder or co-exposure to the mixture, whereas exposure to benzo(a)pyrene alone mainly modified structural proteins. The entire dataset suggested a Trojan-horse mechanism involved in the biological impacts on *Danio rerio* embryos especially due to different bioaccumulation pathways and cellular targets.

TH037
Effects of agro-pesticide cypermethrin on haematology of a freshwater catfish Mystus cavasius
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Cypermethrin is widely used as a pesticide in agriculture of Bangladesh. Since most of the agricultural lands of the country are in the floodplains, pesticides are readily leached into immediate aquatic habitat. Gangetic Mystus, *Mystus cavasius*, is a small freshwater catfish commonly available in the inland freshwater habitats close to agricultural lands. Therefore, a short term definitive exposure experiment was conducted to evaluate changes in hematological parameters of *M. cavasius* exposed to lower to higher concentrations of the synthetic pyrethroid pesticide cypermethrin. Adult female and male of *M. cavasius* were exposed to three triplicate concentrations of cypermethrin- 4 μg/L, 8μg/L and 16 μg/L and control (0 μg/L) for a period from Late June to Late August 2015; 40 fishes (20 female and 20 male) each per 200-L cement cistern were reared. Monthly sampling of blood glucose, haemoglobin (Hb), red blood cells (RBC) and white blood cells (WBC) were done. In both female and male, blood glucose levels increased significantly due to toxicity of cypermethrin. Blood glucose level (Mean ±SE) in control female were between 5±0.59 to 5.53±0.53 mmol L^{-1} and in treatment from 6±0.15 to 10.13±0.53 mmol L^{-1} and in male it ranged from 4.9±0.25 to 5.3±0.57 mmol L^{-1} in control and from 5.73±0.14 to 9.6±0.47 mmol L-1 in treatment. Blood Hb levels in both female and male dropped significantly due to exposure to cypermethrin. Hb (Mean ±SE) in control female ranged from 8.6±0.34 to 9.5±0.59 g dL^{-1} whereas it ranged from 4.6±0.31 to 7.86±0.29 g dL^{-1} in treatments. In male it ranged from 9.06±0.43 to 9.8±0.75 g dL^{-1} in control and 4.8±0.41 to 8.2±0.30 g dL^{-1} in treatments. RBC count also dropped significantly as an influence of cypermenthrine exposure. In female, monthly RBC levels (×106 mm-3; Mean ±SE) ranged from 0.59± 0.06 to 1.88± 0.95 in treatments compared to 2.03±0.36 to 3.27±0.03 in control. In male, the value ranged from 2.48±0.014 to 2.61±0.06 in control and between 0.38±0.03 to 1.88±0.36 in male. WBC counts elevated in most of the female and male fishes exposed to different concentrations of cypermethrin. These results indicate that hematological parameters may be useful as a diagnostic test for cypermethrin exposure in aquatic animals and calls for the limited and cautious use of cypermethrin.

TH038
New endpoint of tail length in fathead minnow embryo-larval tests
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Standard seven-day fathead minnow larval survival and growth tests are useful for the detection of acute toxic effects of chemicals and effluents. We have extended the test adding 5 days at the beginning (egg stage) and 9 days at the end (late larval early juvenile stage). The 21-day embryo-larval exposure starts with fertilized fathead minnow eggs, and continues through hatching, sub-sampling of the larval fish at 9 days post-hatch (dph), and ending at 16 dph. We have used it to study the effects of polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, and oil sands-related compounds, as well as pure chemicals (azo dyes and substituted phenyl amine antioxidants) tested as part of Canada's Chemicals Management Plan. The extended embryo-larval fish test can be used with waterborne chemicals/samples and sediment exposures. One recent addition to the test was the incorporation of tail length in larval fish at 9 and 16 dph as an endpoint. A series of PAH-containing sediments from one Great Lakes Areas of Concern (Randle Reef in Hamilton Harbour, Hamilton, Ontario) were assessed in the 21 day test. While only one of 12 sites sampled was overtly toxic, 4 sites reduced the growth of larval fish. Of these reductions, most of the decreases in overall length of larval fish at 9 and 16 dph were caused by smaller tail lengths. In cases where sediment exposure decreased larval fish length by 9-20 %, tail length was decreased by 36-54 % compared to control sediment-exposed fish. Similar reductions in tail length were seen in embryo-larval exposures to other river sediments from other Areas of Concern (such as the Saint Mary's River in Sault Ste. Marie, Ontario) and sediments from the oil sands area of Alberta that naturally contain alkylated PAHs and PAHs. The measurement of tail length provides an easy-to-measure and potentially sensitive endpoint which could be included in the 21 day fathead minnow embryo larval test (or other larval fish tests).

TH039
Interaction of rainbow trout CYP450 with pharmaceuticals present in aquatic environment
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This *in vitro* study examined the ability of several human pharmaceuticals to modulate hepatic P450-mediated monooxygenase activities in hepatic microsomes from rainbow trout. Following human pharmaceutical found in aquatic environment were included into the study: diclofenac, sulfamethoxazole, tramadol, carbamazepine, venlafaxine and nefazodone. Two reactions, 7-ethoxyresorufin-O-deethylation (EROD) and benzyloxy-4-trifluoromethylcoumarin-O-debenzyloxylation (BFCOD), were used as markers for trout hepatic CYP1A, and CYP3A activities, respectively. Hepatic microsomes were exposed to six concentrations of each pharmaceuticals, including environmentally relevant concentration (0.001 to 100 µM). Neither EROD nor BFCOD activity were affected by environmentally relevant concentration of the tested compounds. Among six tested compounds, nefazodone was found to be a non-competitive inhibitor with IC50 = 16.1 and 12.0 μM with and without pre-incubation step, respectively. Carbamazepine was able to inhibit BFCOD activity to approximately 20%, while presence of nefazodone in the incubations displayed bi-phasic inhibitory pattern of BFCOD. The nefazodone inhibited BFCOD activity (49% inhibition) at 10 µM. To our knowledge this is the first report on the interaction of nefazadone and carbamazepine with rainbow trout CYP450 system. Keywords: diclofenac, carbamazepine, venlafaxine and nefazodone
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TH040

Ultra Sensitive Cyprinid Vitellogenin ELISA: A new tool allowing accurate determination of vitellogenin in male and juvenile fish

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In the past the determination of vitellogenin in fish was carried out in blood and homogenate samples limiting the use of vitellogenin determination in many aspects. Recently, different studies have shown, that vitellogenin can also be measured in epidermal mucus of fish allowing a repeatable, non-invasive sampling. This new method opens the possibility to study vitellogenin concentrations over time or over treatment even in small juvenile fish. For this purpose we specifically developed a very sensitive Vitellogenin ELISA for Cyprinids. This sandwich ELISA requires two immune incubations, first with the capture antibody overnight and second with the detection antibody for 4 hours both at room temperature. At the end the colour reaction is measured at 450 nm and 4-parameter curve fit should be used for automatic data reduction and calculation. The linear standard range is between 0.025 and 2.00 ng/ml (undiluted samples) covering an OD range between appr. 0.05 and 2.0 at 450 nm. The lower limit of detection (LLD) was calculated to be 0.002 ng/ml. The intra- and inter-assay CVs were between 3.3-5.1% and 3.5-5.5%, respectively. Spiking recovery studies in mucus of untreated zebrafish and untreated goldfish resulted in mean recoveries of around 102% and 107%, respectively - linearity studies in zebrafish and goldfish show variations between 86-109% and 98 and 114%. The newly developed ELISA for high sensitive determination of vitellogenin allows an accurate and reliable determination of vitellogenin in the range of 0.025 ng and 2 ng/ml. With this new assay, vitellogenin level can be accurately determined even in male and juvenile cyprinid fish.

TH041

The use of different histochemical staining as an enrichment of histopathological evaluation of the toxicity of chemicals in the fish - the impact on skin.

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Introduction: Toxicity tests on fishes are conducted in order to evaluate possible risks of chemicals (e.g. drugs or pesticides) to similar species and to determine the criteria for water quality. They are, in correlation with tests carried out on other species, one of the steps in the registration process. The methodology of these tests, given in the form of guidelines, is strictly defined by regulatory bodies such as the Organization for Economic Cooperation and Development (OECD), the European Medicines Agency (EMA), or the Environmental Protection Agency (EPA). However, for scientific purposes these procedures can be modified, and adjusted to the needs of researchers. **Materials and Methods:** Experiments were performed on fishes representing three species: Rainbow trout (*Oncorhynchus mykiss*) [n=52], Zebrafish (*Danio rerio*) [n=45], and European carp (*Cyprinus carpio*) [n=17]. Animals were treated with various chamicals (i.e. biocydes, herbicydes or pesticides). A histopathological examination of the skin from treated and control animals were conducted. Samples were collected, fixed in 10% formalin, embedded in paraffin, stained with haematoxylin-eosin (HE, routine), iron hematoxylin-picric acid-acid fuchsin (van Gieson, colagen differentation), iron hematoxylin-periodic acid-Schiff reagent-alcian blue (AB-PAS, detection of polysaccharides, mucosubstances and mucins in tissues), iron hematoxylin-acid fuchsin-phosphomolybdic acid-methyl blue (Masson's trichrome, connective tissue differentation), silver nitrate solution (AgNORs, silver-binding nucleolar organizing regions) and examined under a light microscope. Samples from treated animals were compared to controls from the same experiment. **Results:** Although all staining brought additional data, it is AB-PAS (showing changes in the pH of secreted mucus and also qualitative and quantitative changes in the mucous cells of the epidermis) and AgNORs (showing damage and regeneration ability of epidermis) proved to be most useful. However because of cost and time-consuming to AgNORs method it seems that AB-PAS staining supported by HE is the best approach for histopathological evaluation of the skin of fishes. **Conclusions:** Regardless of whether tests are carried out for regulatory or scientific purposes, one of the key steps is to obtain reliable detailed results, and a histopathological examination enriched with additional staining is a very valuable tool to assess the effect of chemicals on fish.

TH042

Assessment of chemical biotransformation using the fish in vitro metabolism assay: A fish species comparison.

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In vitro chemical biotransformation has has gained acceptance among the scientific community. However, there are many gaps in understanding how different species biotransform environmental chemicals. Fish are the most diverse group of vertebrates; are found in variety of aquatic habitats and display a wide range of feeding modes and reproductive strategies. Therefore, it is not surprising that there are differences in biotransformation of xenobiotics among fish species. The overall objective of this study was to compare xenobiotic metabolism of different fish species that inhabit a range of environmental conditions. Rainbow trout has been widely used for *in vivo* and *in vitro* metabolism and bioaccumulation studies. We compared this reference fish to two species of Antarctic fish that live in high dissolved oxygen (DO) concentrations and low temperatures (T= -2 to 4°C), alligator gar that inhabit higher temperature waters (T= 20 to >30°C) with low DO and brown trout, a close relative to rainbow trout. All fish were euthanized and livers perfused with a buffered clearing solution (pH = 7.8), excised, homogenized, centrifuged to obtain the liver S9 sub cellular fraction and stored at -80°C. The liver S9 fractions of the different species were used in metabolism studies of pharmaceuticals (diclofenac, propranolol and diphenhydramine), a PAH (pyrene), and to determine conventional enzyme activities (EROD, GST). Our results indicate that metabolism of the chemicals varied among species. Although, alligator gar EROD and GST activities were similar to rainbow trout, the metabolism pattern of alligator gar was different from rainbow trout and Antarctic fishes. The most striking difference was observed in the diclofenac metabolism where alligator gar metabolized this pharmaceutical at a much higher rate that the other fish species. Trout and Antarctic fishes appeared to metabolize chemicals differently from alligator gar. Alligator gar diverged from the teleost evolutionary line prior to the teleost genome duplication (TGD) and are therefore genetically more similar to tetrapods than are teleost fish, such as rainbow trout and Antarctic fishes. These results may support previous studies on alligator gar and human gene alignment including genes that are involved in the expression of metabolic enzymes. Unlike teleost fishes, alligator gar may possess unique adaptations to cope with xenobiotics.

TH043

Developing Screening Assays in Zebrafish

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Interest in using the zebrafish embryo/larva as a disease model and assay system for drug and toxicological screening has been expanding rapidly. Many assays include imaging of live and fixed embryos/larvae. Inconsistent orientation of zebrafish can obscure morphological changes and affect image analysis. Existing HTS imaging solutions (e.g. microplate imagers) struggle generating datasets with consistent positioning of images. The new VAST (Vertebrate Automated Screening Technology) BioImagerTM allows automated positioning and imaging of zebrafish larvae. A typical VAST BioImager setup may include a microscope for high-resolution fluorescence image capture. The system loads, positions & rotates a larva to a user selected orientation in the field of view of the camera. Here we present results for assay development using VAST BioImager.

TH044

Ecotoxicological assessment of novel potential formicide: in vitro cytotoxic effects in zebrafish (Danio rerio) hepatocytes

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Farmers' continuous investments in agrochemicals for pest control brings high relevance to development of environmental compounds to reduce use of pesticides with high ecological impact. Amongst, application of toxic baits is highly effective, where the use of metallic complexes can increase bioavailability and amplify biological activities of some substances. However, infiltration and percolation processes may lead chemicals to be released into larger, ecologically important water bodies, influencing surrounding biota. Thereby, *in vitro* cytological biomarkers is essential to rapid assessment of primary effects of xenobiotics. This study evaluates, *in vitro,* cytotoxic effects of a novel potential pesticide ($MgPhen(Hesp)_2$) in zebrafish (*Danio rerio*) hepatocyte cell line (ZF-L). A clear impact on ZF-L cell population was noted 24 h after $MgPhen(Hesp)_2$ exposure evaluating confluence/morphology and cell density as well as MTT and neutral red analysis. Otherwise, apoptosis and necrosis mechanisms were not observed, as well as no differences found in LDH leakage and Trypan blue viability assessments. Cell death pathways still belongs to a research field full of new discoveries. Overwhelming damage of cellular apparatus can lead to passive death mechanisms, as much as active processes such non-apoptotic and non-necrotic responses could be triggered. However, $MgPhen(Hesp)_2$ concentrations ($0.1\ \mu g.L^{-1}$ up to $1\ mg.L^{-1}$) may have its toxic effects mitigate after longer periods (96 h), enabling cellular population to start recovery processes. Elucidating pathways affected through different assays is a crucial step, since different concentrations and exposure times may not exhibit the same effects in a given target but can contribute to a final cellular response. Additionally, assessment of such sensitive biomarkers may serve as early signals to detect xenobiotic effects in organisms prior to manifestation of more severe pathological conditions. Particularly when handling with novel chemicals, assessment of cytotoxic parameters can be use as start to understand mechanisms of cellular responses. Thus, further studies on MgPhen(Hesp)$_2$ effects on the cells are extremely important to point out integration between *in vitro* and *in vivo* responses.

TH045
A Temporal High-Resolution Investigation of the Ah-Receptor Cascade during Early Development of Zebrafish (Danio rerio) after Chemical Exposure
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The fish embryo acute toxicity test (FET) has become a well established method for the assessment and evaluation of chemicals for regulatory purposes. It is not only implemented in an OECD-guideline (no. 236) but can also be used to investigate specific modes-of-action of chemicals during the early development of zebrafish (Danio rerio). In the present study zebrafish embryos were used to investigate the arylhydrocarbon receptor (AhR) signaling pathway in very short time intervals of 4 h from 2 hours after spawning until 118 hpf. The embryos were continuously exposed to different substances inhering various properties (*beta*-naphthoflavone (BNF), polychlorinated biphenyl 126 (PCB126), benzo[a]pyren (BaP)). \nBy using qPCR we measured the expression of all genes involved in the signaling pathway of the AhR. In addition, we quantified the activity of induced biotransformation enzymes (cyp) after exposure using a kinetic EROD-assay. In qPCR, we saw that the cyp-genes were regulated after exposure to the tested chemicals. For PCB126 the gene expression pattern is alternating with several maxima, maybe due to the persistency of the compound. All the co-factors of the pathway (HSP90ß, ARNT1b, ARNT1c, AIP, AhR-Rb and AHR2 itself) remained unaffected. By contrast, after BNF exposure gene expression was significantly raised after 12 h of exposure, climaxed around 28 h and stayed on a lower level until the end of exposure. This observation is most likely due to the fact that BNF was biotransformed and thus induced transcription only in the beginning of exposure. As for PCB126, the other co-factors did not display alternated expression after BNF exposure. qPCR data of BaP will be complete soon. Regarding activity of the cyp proteins, PCB126 caused an elevation beginning at 60 h, whereas BNF did not alter enzymatic turnover. This supports the assumption that cyp1a also possesses a physiological role during the early development of the zebrafish. Its basal activity might be sufficient to metabolize BNF whereas PCB126 is at 60 h as potent as before and therefore causes increased activity. Experiments with BaP are in progress. \nOur results provide a new insight into the regulation of the AhR-pathway and show that it is very important to consider the time slot of exposure when performing biotests like the FET to interpret results properly. The authors thank the German Environmental Foundation (DBU).

TH046
Ontogeny of steroid and thyroid hormone metabolism gene transcription during zebrafish embryo-larval development
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The zebrafish has an impressive range of possible applications as a vertebrate model in both fundamental and applied research. It is increasingly used for incorporating different technologies in understanding the toxicity pathways of various chemicals. The Fish Early Life-Stage (FELS) Toxicity Test (OECD TG 210) is one of the primary ecotoxicological testing guidelines. Gene transcription analyses during the first 32 days of normal development offer us a dynamic picture and may provide us additional information for ecotoxicological tests. Here we describe the gene transcription profiles of the thyroid and steroid hormone synthesis machinery and associated receptors, during the first 32 days of zebrafish development, which has never been done so far. We isolated RNA at 25 time points between 0 and 32 days post fertilisation of zebrafish development covering the most important events during the embryonic and larval stages.We quantified mRNA levels of 20 genes involved in the steroidogenic pathway and 9 genes important for the thyroid systemusing QPCR. Our results show that mainly the enzymes at the beginning of the steroidogenesis pathway are maternally transferred. This suggests an important role of steroid hormones in programming the earliest stages of zebrafish development before the embryo's genome is activated around 3hpf (hours post fertilisation). Further, distinct transcriptional patterns of estrogen receptors are noticed during development. *Esr1* is abundantly transcribed from the time of embryonic genome activation and transcription of *esr2b* increases during the formation of the immature gonad. High transcript levels of *esr2a* due to maternal transfer soon drop and increase later during gonad differentiation. Interestingly, transcription *of isozymes involved in steroidogenesis differs.*Possibly, one is required for early development, whereas the other is important later during development. Transcription of *dio1, dio3a and dio3b* increases at 120hpf. This may be related to inflation of the swim bladder around this time point. *Dio2* start to be abundantly transcribed only after 12 days. These results will improve our fundamental understanding of the role of steroid and thyroid hormones during early life stages of the zebrafish. Information we provided here will be used as a reference dataset for the development of new testing methods targeted at identifying various endocrine disrupting compounds which may act by altering these transcriptional patterns.

TH047
Behavioral analysis and toxicoproteomics profiling of mianserin exposure of zebrafish
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Pharmaceuticals are widely used by humans, for food production or for veterinary purposes, but they may also enter the environment. Unfortunately, many pharmaceuticals have unknown mode of actions in the different environmental niches. Especially neuro-active drugs are of particular concern when acting on non-target species as the neural system is essential for the regulation of various physiological processes and behaviors. The occurrence of antidepressants in surface waters may lead to reduced anxiety of fish, which affects their abilities to deal with predators. In this research we will study effects of the pharmaceutical pollutant mianserin in aquatic environments, on different levels of complexity. In this respect, we will use the zebrafish (*Danio rerio*) which is known as a valid ecotoxicological model. We aim to study altered zebrafish behavior using 3D video tracking as a consequence of exposure to the psychoactive drug mianserin. Doing so, we found a significant effect of swimming angle, as well as the average swimming speed and the time that the fish spend in upper, middle and lower zone in the aquarium. Next, by adopting a differential proteomics approach, we aim to reveal mechanistic information of toxicity at the molecular level, or at least aim to provide a picture of (biochemical) pathways that are affected. While previous proteomics studies in ecotoxicology mainly employed labor-intensive gel-based methods, we explored the use of gel-free strategies. Neuroproteomes of individual brains of exposed zebrafish were compared with their non-exposed controls. Proteins from the entire brain region of the zebrafish were extracted using standard protocols and the resulting proteomes were treated with trypsin to yield thousands of small peptides. A tandem mass tag (TMT) labeling method was used to analyze and quantify six different samples (e.g. three control samples and three samples from exposed fishes) in one single nanoLC-MS experiment to obtain differential proteome maps. Finally, we aim to correlate obtained behavioral parameters with molecular fingerprints from differential proteomics datasets to obtain mechanistic and functional insights underlying aversive effects of exposures to mianserin.

TH048
Neurodevelopment related transcriptome alterations in zebrafish embryo after exposure to valproic acid
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Autism is a complex neurodevelopmental disorder characterized by deficits in social interaction, impaired communication, and repetitive behavior. The prevalence of autism spectrum disorder has recently been increased in Asia, Europe, and United States. Valproic acid (VPA) is used as an anti-epileptic drug and mood stabilizer. However, prenatal exposure to VPA has been linked to the incidence of the autism spectrum disorder. Though VPA has been used as a chemical to induce autism in many experimental studies, the mechanism of VPA is still unclear. Transcriptome analysis would be helpful to understand the underlying mechanism of VPA for autism spectrum disorder. In this study, therefore, we conducted transcriptome analysis using a Next Generation Sequencing approach and observed behavioral (total travelled distance and actively moved duration) and developmental (hatching rate, time to hatch, and malformation) changes in early developmental stage zebrafish after VPA exposure (0, 12.5, 25, 50 and 100 μM). Hatching rate was significantly reduced at 100 μM and time to hatch was significantly increased at 50 μM of VPA exposure. In addition, total travelled distance and actively moved duration of zebrafish was decreased at 50 μM of VPA exposure. In transcriptomic analysis, differentially expressed genes were associated with neurogenesis, nervous system development and sensory perception etc. Specifically, transcripts in GABA receptor modulator, dopamine receptor, parvalbumin were significantly reduced in zebrafish exposed to all the concentration of VPA. Overall, our transcriptome data imply that VPA exposure caused transcriptome changes related to various neurodevelopment in early development stage of zebrafish. The transcriptome profiling of zebrafish embryo after exposure to VPA are also expected to improve our current understanding of the molecular mechanism of VPA. This study could also contribute to the zebrafish model development for autism research.

TH049
Analysis of the relationship between effects in the locomotor activity and

morphological alterations induced by biocides in zebrafish embryos
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The developing zebrafish embryo is increasingly used as an alternative animal model for evaluating different toxicities. The advantages of the zebrafish are mainly their low cost and ease of maintaining and breeding. Moreover, much of the research and assays conducted in zebrafish have been carried out in larvae to take advantage of zebrafish fecundity, small size of larvae, easy of handling and transparency. Agrochemicals are extensively used worldwide. Their potential as developmental toxicity is usually analyzed. However, some of them might be also linked to neurological disorders. Moreover, neurotoxicity can be induced at concentrations where neurotoxicants appear to be safe in developmental toxicity assays. In this study, we tested six biocides; myclobutanil, penconazole, dieldrin, methoxychlor, difenoconazole and triclosan. Firstly, we carried out developmental toxicity assay. For this purpose, we selected wild type embryos at 3 hours post fertilization (hpf) and treated with each biocide. After 96 hours of exposure, we evaluated the morphological effects found during the embryonic development. Additionally, we evaluated the behavioral alteration that could be induced by tested biocides. We treated wild type embryos at 5 days post fertilization (dpf) during 2 hours at five concentrations chosen based on the results of the developmental toxicity assay. At least four of these five concentration were lower than concentrations with morphological alterations. Finally, they were placed in the Daniovision automated tracking system powered by Ethovision (Noldus). In conclusion, developmental toxicity assay should be complemented with behavioral assay conducted at concentrations where no morphological alteration was detected. Therefore, the resulting neurodevelopmental toxicity assay would be a test with higher sensitivity. In addition, the assay supports the 3R-principles and provides an opportunity for *in-vivo* primary screening.

TH050
Toxic effects of kaolin residues on embryos, juveniles and adults of zebrafish Danio rerio
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Kaolin is a clay used as a raw material in the ceramic industry and for the production of paper, cosmetics and medicines. The main impact that mining kaolin, are deforestation and the contribution of waste into water systems, causing changes in water quality. In this work were evaluated the toxic effects of kaolin particles, which are supplied to an aquatic system as suspended solids, in embryos, juvenile and adults of zebrafish. Bioassays were performed in which fishes were exposed to 6 kaolin concentrations (0.01, 0.1, 1.0, 10, 25 and 50 mg L^{-1}), plus a negative control for 48 hours in embryo and juvenile tests and by 96 Hours in assays with adults. In the bioassays LC_{50} (lethal concentration 50) was determined and a comparison of LC_{50} was performed to evaluate the sensitivity of the embryos, juvenile and adults. In addition, the respiration rate, excretion rate and the degree of stress in which the organisms were found, determining the O:N index were evaluated. The most sensitive organisms to the kaolin particles were the fry (LC_{50} = 4.8 mg L^{-1}) and the least sensitive adult (LC_{50} = 176 mg L^{-1}). An increase in respiration rate and excretion rate was observed in the first 24 hours of exposure to kaolin. Subsequently, a decrease in respiration rate in juveniles and adults it was observed. The degree of stress was high in bioassays with juveniles and adults. Because the LC_{50} values obtained in embryo and juvenile bioassays are below the maximum permissible limits of NOM 001-Semarnat for suspended solids discharged into aquatic systems (40 to 60 mg L^{-1}), it is important to continue conducting research and monitoring to detect responses indicating possible damage to fish stocks to avoid irreversible deterioration of populations in the medium and long term.

TH051
pH-Dependent Effects of β-Blockers in Zebrafish Embryos
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Many pharmaceuticals can be detected in the aquatic environment. Even low concentrations are potentially of concern due to their design towards specific drug targets, which are evolutionary conserved in aquatic organisms, especially in fish. β-adrenergic receptor antagonists (ß-blockers) are amongst the most used pharmaceuticals and have been detected in surface waters at concentrations up to a few μg L^{-1}. Effects of ß-blockers have been observed in different fish species and can be related to the pharmacological mode of action. ß-Blockers are weak bases with acidity constants mainly influenced by their secondary amine group. Therefore they are protonated at environmental pH conditions with tendency to shift to their neutral form at more alkaline pH. The neutral species can pass the biomembrane more easily than the corresponding ionic species. This difference in uptake might result in enhanced toxicity in the presence of neutral fractions. Here we studied the influence of pH on toxicity of four ß-blockers in zebrafish embryos. The zebrafish *Danio rerio* is a popular model organism for fish and its 96h standardized fish embryo toxicity test (OECD TG 236) is considered as an *in-vitro* assay. In accordance with the OECD test the β-blockers Atenolol, Metoprolol, Labetalol and Propranolol were tested at pH values not toxic to the embryos ranging from pH 5.5 to 8.6. Additionally to lethality we evaluated assays on changes in swimming activity and heartbeat after β-blocker exposure, using the Locomotor Response (LMR) method and the Vertebrate Automated Screening Technology (VAST) for high throughput imaging. The test protocols were adapted to stabilize the pH values with non-complexing buffers (MES, MOPS, HEPPS, TAPS). The results indicate significant effects of Metoprolol, Labetalol and Propranolol on heartbeat and swimming activity. All 3 pharmaceuticals showed an enhanced toxicity with an increase of neutral fraction at more alkaline pH, with lethality of Propranolol showing a 100 fold difference between LC_{50} at fully cationic state at pH 7 and slight shift to neutral state at pH 8 (3 %). Atenolol did not show any effects at concentrations up to 10 mM. The observed effects support the hypothesis that the uncharged form, with its higher bioavailability, is driving the enhanced toxicity and highlights the importance of pH-dependent aquatic toxicity for ionizable pharmaceuticals.

TH052
Effects of sublethal dietary exposure of Microcystis aeruginosa and the toxin microcystin-LR on target-gene expression profiles, histopathology and gut microbiota in adult zebrafish
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Blooms of toxic cyanobacterial are a worldwide problem that affects freshwaters. Among the Cyanobacteria that produce toxic blooms, *Microcystis sp*. release hepatotoxins known as microcystins (MCs). Ingestion of food or water containing *Microcystis* and/or microcystins is a relevant route of exposure, and the consequences of exposure may affect gut physiology, immune system function, and overall organism health. Our objectives were to investigate effects of dietary exposure to *Microcystis aeruginosa (M. aeruginosa)*, or microcystin-LR (MC-LR) on target gene expression profiles, histopathology, and gut microbiota community in adult zebrafish. Adult zebrafish (males and females, age 6 months) fed twice a day (3% body weight per day) food that was prepared in lab with the following dietary treatments: lyophilized *M. aeruginosa* or MC-LR for 14 days at three concentrations (0, 5 and 10 μg MC-LR/g food). Three tanks per treatment used as replicates, each experimental fish tank had eight fishes (mixed sex), and fish were sampled for different endpoints at 6, 24, 48, 96 hours and 14 days. At the end of exposure, there were no significant effects on fish growth or condition, and no fish died during the experiment. Investigation of gut microbiota is underway [via Terminal Restriction Fragment Length Polymorphism (T-RFLP)], and histopathology of intestine and liver are in process. Changes in expression of target genes by real time quantitative polymerase chain reaction (Q-PCR) for the following genes: catalase (*CAT*), superoxide dismutase1 (*SOD1*), glutathione peroxidase (*GPx*), glutathione-S-transferase (*GST*), cytochrome P450 (*CYP1A*), protein phosphatase (*PPP1*), and vitellogenin (*VTG)* are underway in intestine and liver samples. We anticipate that these results will enhance understanding of the molecular responses, and the changes in microbiota community and histopathology in adult zebrafish during the progression of dietary exposure to a HAB caused by *M. aeruginosa*. <strong>**Key words:** Sublethal dietary exposure, *Microcystis aeruginosa*, microcystin-LR, Zebrafish gut microbiota.

TH053
Mixture-specific gene expression in zebrafish (Danio rerio) embryos exposed to perfluorooctane sulfonate, perfluorohexanoic acid and 3,3',4,4',5-pentachlorobiphenyl.
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Perfluorooctane sulfonate (PFOS) and Pentachlorobiphenyl 126 (PCB126) are persistent organic pollutants withdrawn from the market but remaining of high concern because of their environmental persistence, bioaccumulation and toxic properties. Additionally, the amphiphilic properties of PFOS suggest a role in increasing cell membrane permeability and solubilizing chemicals. This is of particular relevance in risk assessment since under regular environmental conditions organisms are exposed to multiple chemicals. The present study aimed at investigating whether PFOS and one substitute, Perfluorohexanoic Acid (PFHxA), are capable of increasing PCB126 toxicity. For this purpose, zebrafish embryos were exposed in semi-static conditions to 23 nM of PCB126, 50 μM of PFOS and 50 μM of PFHxA alone or in binary and ternary mixtures. Embryos were sampled at 24, 30, 48, 54, 72, 78 and 96 hours post fertilization (hpf) for gene expression analyses using RT-qPCR. Expression of genes involved in metabolism of xenobiotics (*ahr2*, *cyp1a*), oxidative stress (*gpx1a, tp53*), lipids metabolism (*acaa2, osbpl1a*), and epigenetic mechanisms (*dnmt1*, *dnmt3ba*) was investigated. PFHxA and PFOS alone did not induce any significant modification in gene expression. *Cyp1a* and *ahr2* expression were significantly induced by PCB126 alone and in the different mixtures. However, after 72 and 78 h *cyp1a* expression was significantly lower when embryos were co-exposed to PCB126+PFOS+PFHxA. Significant

upregulation of *gpx1a* occurred after exposure to PCB126+PFHxA and to the ternary mixture at 30 and 48 hpf. Besides, embryos appeared more sensitive to the ternary mixture at 78 hpf: *acaa2* and *osbpl1a* were significantly downregulated; *dnmt1* was significantly upregulated. In conclusion, the present results show that PFOS and PFHxA are modifying PCB126-induced gene expression in zebrafish embryos, suggesting that they are able to alter its toxicity. However, further investigations are required to gain a better understanding of the mechanisms leading to a decrease of *cyp1a* induction in presence of the three chemicals.

TH054

Interference of hepatotoxicity with endocrine activity in fish

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Vitellogenin (VTG) has been established as key biomarker for the diagnosis of endocrine disruption in fish, which is used in the OECD test guidelines 229, 230 and 234. A reduction of VTG production is usually associated with androgenic or anti-estrogenic activity, whereas an increase of VTG production is regarded indicative for the presence of estrogenic compounds. However, the production of VTG may not only be modified by typical endocrine-related pathways, but also through non-endocrine-mediated processes. In particular, hepatotoxicity, i.e. toxicant-induced impairment of liver structure and function, can influence the VTG biomarker, as it is synthesized in the liver. A false VTG result in a screening assay would unnecessarily trigger very labour-, time- and cost- intensive higher tier-testing, as it would increase the number of fish used in experiments. Therefore, an intimate understanding of the interplay between primary endocrine-related and non-endocrine-related pathways influencing VTG production is crucial for the avoidance of false-positive diagnoses. The present project is driven by the hypothesis that hepatotoxicity may positively or negatively interfere with VTG production, which is used as biomarker of endocrine activity in current OECD test guidelines. Consequently, the project has been designed to: identify scenarios, where liver toxicity may affect the induction, synthesis and secretion of VTG from hepatocytes in small fish models; develop a set of diagnostic tools to distinguish liver toxicity-mediated modulation of VTG production in fish from primary endocrine effects. Zebrafish (*Danio rerio*), one of the most commonly used small fish model species, is used in OECD guideline 229 (“Fish Short Term Reproduction Assay”) type assays to address the above-mentioned aims. Hepatotoxicants with different modes of action, namely acetaminophen, isoniazid, valproate and dinitro-*O*-cresol (DNOC), are applied as model compounds to induce different effects on the liver of exposed fish. Endpoints recorded include: (1) histopathology of liver and gonads to assess liver structural toxicity, (2) measurement of VTG and hormone levels, (3) determination of serum liver toxicity markers (alanine aminotransferase) to assess liver functional toxicity, (4) transcriptomic analyses (RNA-seq) of genes involved in energy metabolism and (5) respirometric measurements of the energy budget to assess liver functional toxicity.

TH055

Coexposure effects of 17α-ethinylestradiol in the presence of micro- and nanoplastic on embryo development and larvae behavior of Danio rerio

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The entry of endocrine disruptor chemicals (EDCs) and microplastic in aquatic systems became a global problem. Due to mechanical and physical destruction, huge plastic particles are shredded into particles with sizes smaller than 5 mm which are called microplastic particles (MP). They can function as vectors for chemicals and co-pollutants, affect the bioavailability and influence the entry and transfer of chemicals in food chains. The focus of this study was the analyzation of the 'trojan horse effect' reagarding to the changes of the behavior of *Danio rerio* larvae. The coexposure effects of 17alpha-ethinylestradiol (EE2) with a concentration of 2 and 20 microgram/L and MP with a concentration of 1 milligramm/L on the development, behavior, the antioxidant enzyme activity and the gene expression of *Danio rerio* was analyzed in this study. Changes in the swimming behavior of the zebrafish larvae were tested with the Danio Vision(R) observation chamber. In the coexposure treatments of microplastic and EE2 a significant decrease in the swimming distance of zebrafish larvae could be detected. The analyzation of the coexposure effects on oxidative damage were done with glutathione reductase (GR), glutathione peroxidase (GPx) and catalase (CAT) which showed an upregulation of the enzyme activity in all cases. The effects on gene level were tested with a real-time polymerase chain reaction. *Gfap, alpha1-tubulin* as two genes for neurodevelopment monitoring and *zfrho* and *zfgr* as two genes which can reflect the visual damage level. For all genes significant differences between the tested treatment and the control group could be detected. The relevance for the ecosystem and the environmental research is the combinative analyzation of the effects between EDCs and MP. The co-exposure between both components influence and change the bioavailability and toxic effects of EE2 on larvae of *Danio rerio* which could be a hint for changes in other biological processes, too. In further studies the effects of cellular and molecular level will be analyzed to find out how processes on this level will be changed and how they influcence the processes on other ecosystem levels.

TH056

Effects of bisphenol SIP on HPG axis and reproduction in zebrafish (Danio rerio)

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4-hydroxyphenyl 4-isoprooxyphenylsulfone (bisphenol SIP, BPSIP) is widely used as dye developers in thermal paper products as an alternative compound of bisphenol A (BPA). Previous study reported that cashiers have greater levels of BPSIP in urine or blood samples than non-cashiers. BPA and related compounds are well-known as weak estrogen agonists, however endocrine disruption potential of BPSIP have not yet been elucidated. In the present study, effects of BPSIP on hypothalamus-pituitary-gonad (HPG) axis and reproduction were investigated using adult zebrafish. Adult zebrafish pairs (*D. rerio*) were exposed to environmentally relevant concentrations (0, 0.5, 5, and 50 μg/L) of BPSIP for 21 days, according to the OECD test guideline 229 with minor modification. The number of eggs spawned were recorded daily, and the medium was renewed every 2 d. The effects on organism level (number of eggs), organ level (gonad-somatic index), hormone level (sex steroid hormones) and gene level belonging to the HPG axis were invetsigated. After exposure to BPSIP, decreased trend of egg production was observed. Gonado-somatic index was significantly increased in male fish exposed to ≥5 μg/L BPSIP. Interestingly, the adverse effects of BPSIP on organ, hormone, and gene level biomarkers were sex dependent, with males being more sensitive than females. In male fish, significant increase of 17*β*-estradiol (E2)/testosterone (T) ratio was observed at ≥5 μg/L BPSIP along with up-regulation of *cyp19a* transcripts. Concentrations of plasma T were significantly increased in females exposed to 50 μg/L BPSIP along with up-regulation of *cyp17* transcripts. These results are in good agreement with those reported for bisphenol S, while precise mechanisms on endocrine disruption were different between two chemicals. The results of the present study showed that exposure to low level BPSIP could affect regulatory systems of HPG axis and reproduction in zebrafish at environmentally relevant concentrations. Acknowledgement - This study was supported by the National Research Foundation of Korea (Project NRF-2015R1D1A1A01056628).

TH057

Response of zebrafish and Japanese medaka to imidacloprid: a comparative study

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Neonicotinoids are among the most produced and used insecticides and are frequently found in freshwater with concentrations ranging from ng to *μ*g/L. These molecules are small and very soluble in water. This given, they are likely to pass the chorion and thus easily enter embryos of fish. It is known that these compounds impact on insects, including bees, in terms of toxicity and behaviour, but whether similar impacts could be observed in fish is not yet known. The aim of this study was to investigate effects of exposure to imidacloprid, one of the most used neonicotinoids, on the development and behaviour of two common fish lab species: Zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*). Fish were exposed during 5 (zebrafish) and 13 days (medaka) to 0, 0.2, 2, 20, 200 and 2000 *μ*g/L imidacloprid by aqueous exposure, matching the respective time from fertilization to emerged larvae for the two species. At similar developmental stages, survival, hatch, growth, morphology, behaviour and histology were examined. No impact on survival was found. A delay for 2 *μ*g/L imidacloprid exposures was observed for hatch in both species. Except for the 20 mg/L exposure level, imidacloprid led to hypoactivity in both species. A striking difference was found in morphology: a high number of deformities were noticed for medaka whereas almost none were found in zebrafish. Yolk and bone oedemas started to be noticed at 20 *μ*g/L after hatch, jaw deformity and lordosis/scoliosis increased with concentration level. An increase of haemorrhage was found at the highest concentration (2000 *μ*g/L). In order to shed light on the observed species differences, we intend to investigate the uptake of imidacloprid into the fish embryos and its biotransformation.

Determining population relevance of ecotoxicological effects (P)

TH058

Deriving ecologically relevant endpoints for wild mammal risk assessment

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The risks to wild mammals from potential exposure to plant protection products must be assessed in accordance with Regulation (EC) 1107/2009. The risk assessment is currently performed according to the bird and mammal guidance document (EFSA Journal 2009; 7(12): 1438). Under this guidance, the actual protection goal is to clearly establish no visible mortality and no long term repercussions for abundance and diversity, with the surrogate protection goal being to make mortality or reproductive effects unlikely. In the Tier I risk assessment for wild mammals, the lowest available no observed adverse effect level (NOAEL) from the developmental study or 2-generation rat study should be used. However, these endpoints may not be of ecological relevance for wild mammal populations in terms of addressing the protection goals. This is briefly discussed in Section 4.4 of EFSA (2009), in terms of which *types* of effects are considered relevant for wild mammal populations. However, little guidance is available on how to determine whether the effect is of ecological relevance; instead, a weight of evidence and expert judgement is required. It was recently recommended by Member State ecotoxicology experts (EFSA supporting publication 2015: EN-924. 62 pp.) that ecologically relevant reproductive endpoints should be assessed and agreed during active substance evaluations at EU level rather than during national registrations. The aim of this poster would be to present our experiences in deriving ecologically relevant endpoints for use in wild mammal risk assessments and the factors that require consideration, including the type of effects observed; the magnitude of effects observed; the consistency of dose-response patterns; contextualisation of the effects observed in the critical toxicity study in light of all available data e.g. other chronic toxicity studies, natural variation observed in historical control data, natural variation observed in wild mammal populations; the use of benchmark dose modelling; and consideration of the proposed uses of the active substance relative to the exposure profile experienced during toxicity tests.

TH059
Analysis of relation between bodyweight, survival and reproductive success in Common voles (Microtus arvalis)
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Common vole (*Microtus arvalis*) is one of the best studied small mammals in Europe. Field studies from a large number of investigators provided a substantial amount of data on its ecology, reproductive biology, population genetics and other aspects of this species. Despite this large amount of data, the relevance of body weight on survival and reproduction in adults, sub-adults or juveniles, was never specifically analysed. Marked seasonal changes of body weight have been reported by various researches, but it is unknown if they have an impact on reproductive success or fitness. We therefore aim to provide answers to this fundamental question of common vole ecology by analysing a large dataset from a multi-annual study on common voles (Hahne, 2009), which included life-trapping and genetic genotyping. This analysis focused on seasonal changes of body weight and potential correlations with survival and reproductive success (based on a genetic assignment of offspring).

TH060
Continuous and intermittent exposure to the toxigenic cyanobacterium Microcystis aeruginosa produces different negative effects on the survival and reproduction of Daphnia curvirostris
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Eutrophication has increased due to anthropogenic activities through the rise in discharges of limiting mineral nutrients, such as P and N, promoting phytoplankton growth. Eutrophication produces habitat degradation as consequence of harmful cyanobacterial blooms (HCB) and the release of cyanotoxins, affecting aquatic biota and threatening human health. *Microcystis aeruginosa* (*Ma*) synthetizes and accumulates microcystins (MCs), these toxins are released to the water after the cellular lysis or when the cells are stressed. Because in the natural environment the zooplankters are exposed continuously or intermittently to toxigenic cyanobacteria, and the interaction with MCs could be direct (in the surrounding water), or indirect (though the cyanobacterial consumption), the objective of this study was to assess the effects produced by different exposure forms on the cladoceran *Daphnia curvirostris*. The toxigenic strain *M. aeruginosa* VU-5 was grown in Z-8 medium; the cells were separated from the culture medium by centrifugation. The acute toxicity (48-h) produced by the cells, the aqueous crude extract of cells (ACE), and the exhausted culture medium (CM) were determined; the MCs content in each condition was determined. The effect on the survival and reproduction of *D. curvirostris* of *Ma* cells under continuous and intermittent exposure was determined during 26 d. The LC_{50} for cells was 407,000 cells mL^{-1}; exposure to the ACE and CM produced a lower than 50% mortality. MCs content in cells and in the ACE was 1.6 and 2.1 $\mu g\ L^{-1}$, respectively; in the acute toxicity assays MCs concentration ranged from 0.147 to 0.728 $\mu g\ L^{-1}$. Survival, total progeny, and the number of clutches were significantly reduced with the highest *Ma* concentrations. Continuous exposure to *Ma* cells produced 100% mortality on the 4^{th} day. Exposure to *Ma* cells during 24-h in 48-h cycles produced adult mortality from the 18^{th} day on. When exposure was reduced to 4 h in 48-h cycles, survival was also significantly reduced. Reproduction decreased significantly as the exposure time and *Ma* concentrations increased, with all treatments. The higher toxicity of cells than that of the ACE could mean that MCs absorption is higher in the digestive tract. The episodic exposure to *Ma* cells produced irreversible damages, despite the periods of recovery with healthy food. The form as well as the continuity of *Ma* exposure produced damages, warning about threats to the aquatic biota.

TH061
Effects of long term exposure to imidacloprid on Asellus aquaticus populations
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Imidacloprid is one of the major insecticides which is used worldwide and could affect non-targeted organisms through runoff, leaching, and spray drift into water body after its use. It could harm aquatic organisms since imidacloprid is highly toxic for aquatic insects and several other aquatic invertebrates. Although that *Asellus aquaticus* is not the most sensitive organisms to imidacloprid exposure, they are used in this experiment because they spend their whole life-cycle in the water and are, herewith, also potentially exposed during their whole life-cycle as imidacloprid proved to be very persistent when UV levels are low. The current experiment was conducted to evaluate the effect of imidacloprid on 4 endpoints of *A. aquaticus* (abundance, size distribution, feeding rate, and growth rate) during 120 days of imidacloprid exposure (0; 3; 10; 30; 100; and 300 ng/L). The control treatment consisted of 6 replicates while the imidacloprid treatments were triplicated. Each replicate consisted of a 5L bucket (Figure 1) filled with 20 specimen of *A. aquaticus* with 3 interlocked pairs of male and female while the remaining 14 individuals were selected randomly, while 2 grams of *Populus* leaves, 3 *Elodea* shoots (5 cm each) and a 10x10 cm aluminium mesh were added as food, shelter and/or substratum. The total abundance showed a clear dynamic, after an initial increase in abundance due to reproduction, a gradual decline in numbers due to background mortality was observed. A non-significant lower abundance was found for the three highest treatments. A NOEC of < 3 and 3 ng/L were calculated for the abundance values of the smallest organisms (< 3 mm) at the end of the experiment. This decrease in smaller organisms in all treatments was accompanied by an increase in the feeding rates of the *A. aquaticus* populations on *Populus* leaves and to a lesser extend also on *Elodea*, with NOEC values ranging between < 3 to 10 ng/L for the last few samplings. The results show that imidacloprid will affect the size distribution of *A. aquaticus* at concentrations below European regulatory threshold levels and concentrations regularly occurring in the field. Whether these effects are regulatory unacceptable remains to be evaluated as well as whether they will result in effects on reproductions since our study was too short to observe reproduction. When the effects on reproduction are known, population models can be used to extrapolate the observed effects over generations.

TH062
Macromolecular, morphological and population effects in the cladoceran Simocephalus mixtus exposed to glyphosate (FAENA®)
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Glyphosate is the herbicide most used worldwide. It has been demonstrated that the commercial formulations of pesticides are significantly more toxic than the active ingredients. Exposure to the most popular glyphosate formulations produces negative effects on non-target species, including the aquatic biota. The objective of this study was to evaluate in the freshwater cladoceran *Simocephalus mixtus* the effects produced by glyphosate exposure (FAENA®) on some metabolic biomarkers, the size of the progeny, and the main demographic parameters. The acute toxicity (48 h) was determined; after this, sub-chronic (21 d) and chronic (full life cycle) bioassays were performed assessing the effect of sub-lethal concentrations: 2.19, 2.71, 3.29, 3.64, and 4.06 mg L^{-1} of glyphosate. The obtained LC_{50} was 5.27 mg L^{-1}. Life expectancy at birth, survivorship, and fecundity were significantly affected with the two highest concentrations (3.64 and 4.06 mg L^{-1}) with respect to the control. The increase in glyphosate concentration and exposure time produced a decrease in the net reproductive rate (R_0) and the intrinsic growth rate (r). The size of neonates released in the eight broods recorded in the full life cycle experiment varied among treatments and broods; the body length of neonates in the control ranged from 557 to 565 μm and the body width from 280 to 301 μm; in the treatments, a reduction in body size was documented. In the controls, the content of macromolecules was: protein>carbohydrates>lipids, but the content of proteins and lipids decreased when the concentrations and time of exposure increased, whereas the content of carbohydrates increased with all glyphosate treatments. *S. mixtus* depicted a great sensitivity to the commercial formulation, FAENA®, in all the assessed responses. The information obtained revealed important negative effects of this herbicide on non-target organisms in aquatic systems, both at the biochemical and population levels.

TH063
Determining the population relevance of radiation-induced reproductive

effects in the marine amphipod, Echinogammarus marinus
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The majority of radiation studies in ecotoxicology involve the impacts of acute high doses on individual-level endpoints that are unrepresentative of environmental exposures. Given the emerging importance of radioactive materials as an environmental pollutant, elucidating the impacts of radionuclides at the population level is fundamental to providing a robust risk assessment of the effects of radioactivity on the wider environment. Previous studies have modelled the impact of chronic radiation exposure on population growth in model organisms such as daphnids and earthworms. These studies typically consider female reproductive endpoints such as egg production or time taken to reproduce. Comparatively, the higher-level impacts of a reduction in male fertility have been ignored. There is a need to expand existing studies to more diverse and ecologically relevant groups of organisms. Amphipods are widespread in aquatic environments globally and provide an important link between trophic levels. Previous studies have used dynamic deterministic models to assess the consequences of a reduction in fecundity in amphipods. This study aimed to assess the impacts of environmentally relevant doses of radiation on male fertility and subsequent reproduction in the intertidal amphipod, *Echinogammarus marinus*. Male *Echinogammarus marinus* individuals were exposed to beta radiation from phosphorus-32 at doses of 0, 0.1, 1 and 10 mGy/d and the quantity and quality of spermatozoa assessed using LIVE/DEAD viability staining. Exposed *E. marinus* individuals were paired with an unexposed sexually mature female to assess the consequences of a reduction in fertility on reproduction. Radiation exposure led to reductions in the percentage of viable sperm of up to 11% at 10 mGy/d. A concomitant reduction in the size of broods was recorded at all dose rates. Existing models predict long-term effects of observed fecundity reductions and significant impacts on population success. However, field data obtained within this project suggests abundant aquatic invertebrate populations at Chernobyl. The disparity between model predictions and field data suggests the potential for adaptation over thirty generations of radiation exposure. These results have implications for populations of organisms inhabiting contaminated environments such as Chernobyl and Fukushima and will directly contribute to future risk assessments of the effects of radiation on the wider environment.

TH064
Effects of an aquaculture pesticide (diflubenzuron) on non-target shrimp populations: from lab experiments to population-level endpoints
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The continued growth of marine aquaculture production has presented the industry with environmental and production concerns, of which the ectoparastic salmon lice (*Lepeophtheirus salmonis*) has gradually become a major problem. A commonly used pesticide against this crustacean is diflubenzuron (DFB), which acts as a chitin synthesis inhibitor and thereby interfere with the moulting stages during sea lice development. However, DFB from medicine feed may also affect non-target crustaceans such as the Northern shrimp (*Pandalus borealis*), which is an economically and ecologically important species in Norwegian fjords. Laboratory experiments have demonstrated that shrimp exposed to DFB through fish feed have reduced survival (ca. 60%) compared to control, in both the larval and the adult stages. Moreover, the effects of DFB exposure is more severe under future climate conditions (higher temperature). The aim of this study is to make the information on these mechanistic effects more relevant for risk assessment at the population level. We have developed an age-structured population model representing a Northern shrimp population located in a hypothetical Norwegian fjord containing a fish farm, under both ambient and future climates. Our model is based on thorough knowledge of shrimp biology and clear results on toxicological effects from the laboratory experiments. Nevertheless, extrapolating the individual-level effects to the population level poses several challenges. Relevant information on shrimp populations in fjords is sparse (such as abundances, survival and reproductive rates, and density-dependent processes). The degree of exposure to medicine feed at different distances from aquaculture farms is also uncertain. We have therefore developed a set model scenarios representing different medicine application schemes and different degrees of exposure for the shrimp populations. The purpose of the model is to predict effects of DFB exposure on population-level endpoints such as long-term abundance and age structure, and to assess the risk of population decline below threshold abundances.

TH065
Assessing the spatial distribution of the gastropod Olivella semistriata by using whole-sediment avoidance and recolonization assays
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In situ observations seem to indicate that the spatial distribution of the snail *Olivella semistriata* on the Ecuadorian coast (city of Manta) is influenced by urban discharges. Therefore, contamination could determine habitable areas for snails. Firstly, the present study evaluated the ability of *O. semistriata* individuals to detect local contamination and avoid inhabiting contaminated sediments. Secondly, the ability of snails to recolonize contaminated sediments under recovery was studied. Sediment samples were taken in five points (El Murciélago beach - reference point, El Puerto, La Poza, Río Burro, Los Esteros, and Río Muerto) and evaluated for avoidance and recolonization trials. The tests were performed in a non-forced exposure system in which a contamination gradient was formed by mixing the test sample and the reference sediment. Higher avoidance percentage was observed in the samples of Rio Burro and Rio Muerto. Regarding recolonization tests, the reference sample was always preferred over all test samples. As there is no physical barrier to avoid the displacement of organisms between the studied areas, it is suggested that the absence (visual field observation) of snails in the Rio Burro and Rio Muerto sediments is due to the organisms' ability to avoid those areas. We can conclude that the spatial distribution of the snail may be directly affected by the presence of contaminants in the sediment, by triggering avoidance or even prevent colonization of contaminated areas. Contamination may, therefore, act as a chemical barrier that could cause habitat disruption and isolate populations.
Keywords: *Olivella semistriata*, Habitat disruption, Avoidance, Recolonization

TH066
Direct Blue 15 dye affects differently organisms of different trophic levels. A comparative study with microalgae, cladocerans, and zebrafish embryos
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Global consumption of synthetic dyes approximates 7×10^5 tons per year, and the textile industry consumes about two-thirds of all the world production. About 10 to 15% of the total dye used by the industries are lost during the dyeing process, being released into the aquatic environment through the effluents. The discharge of colored wastes not only produces aesthetic effects in receiving streams but also toxic damages to the aquatic biota. Some dyes, such as Direct Blue 15 (DB15) are elaborated with benzidine, a known carcinogenic compound. Information regarding dye toxicity in aquatic ecosystems is scarce, despite the environmental impacts they produce; therefore, our study was aimed at evaluating the toxic effect of Direct Blue 15 on a bioassay battery consisting of the microalga *Ankistrodesmus falcatus*, the cladocerans *Daphnia magna* and *Ceriodaphnia rigaudi*, and zebrafish *Danio rerio* embryos. *A. falcatus* was exposed to 4, 8, 16, 32, and 64 mg L^{-1} DB15 (96 h at 25°C, continuous illumination of 120 µmoles $m^{-2} s^{-1}$). Cladoceran neonates were exposed to 150, 200, 250, 300, and 350 mg L^{-1} DB15 (48 h at 22°C, light intensity of 120 µmoles $m^{-2} s^{-1}$, and 16:8 h photoperiod). Zebrafish embryos were exposed to 100, 200, 300, 400, and 500 mg L^{-1} (144 h at 26 + 1°C, light intensity of 120 µmoles $m^{-2} s^{-1}$,and 16:8 h photoperiod). *A. falcatus* was the most sensitive to DB15 (IC_{50} = 28.97 mg L^{-1}) of all the tested organisms. The LC_{50} values for *D. magna* and *C. rigaudi* were 223.38 and 284.14 mg L^{-1}, respectively, which could be non-environmentally relevant values. The hatching percentage of *D. rerio* larvae decreased significantly as DB15 dye concentrations increased. Lethal effects, according to OECD guideline 236 criteria (embryo coagulation and no heartbeat), were observed starting with the 200 mg L^{-1}concentration. Results demonstrate that DB15 dye affects mainly primary producers. Lethal effects in zebrafish embryos warns about the potential damages of Direct Blue 15 dye in fish. The DB15 exposure caused toxic effects of different magnitudes on organisms of different trophic levels. According to our results azo dyes must be included in environmental monitoring and regulation programs because they are continually discharged into the aquatic environment.

TH067
A review of the evidence for endocrine disrupting effects of current-use chemicals on wildlife populations
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This review critically examines the data on claimed endocrine-mediated adverse effects of chemicals on wildlife populations. It focuses on the effects of current-use chemicals, and compares their apparent scale and severity with those of legacy chemicals such as DDT, PCBs and TBT, which have been withdrawn from sale or use, although they may still be present in the environment. The review concludes that the effects on wildlife of many legacy chemicals with endocrine activity is generally greater than that caused by current-use chemicals, with the exception of the pharmaceutical ethinylestradiol (EE2) and other estrogens found in sewage effluents, which are causing widespread effects on fish populations. It is considered that current chemical testing regimes and risk assessment procedures, at least those to which pesticides and biocides are subjected, are in part responsible for this

improvement. This is noteworthy as ecotoxicological testing is currently focussed on characterising apical adverse effect endpoints rather than identifying the mechanism responsible for any observed effects. Furthermore, a suite of internationally standardised sensitive ecotoxicological test methods for potential endocrine-mediated effects is now in place, or under development, which should ensure identification and appropriate regulation of substances with these properties.

TH068

Field based ECx - approach to NTA Risk assessment

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At the field level, the risk that exposure to plant protection products may imply to Non Target Arthropods (NTA) is currently addressed through (statistical) NOEC-type study designs. Although the caveats around NOEC-designs have been widely discussed, it is at present the only viable experimental option because the alternative, EC_x-type studies, have not been developed. Here we present a small scale but comprehensive field study using natural NTA populations. The objective was to obtain meaningful dose-effect regressions for a large array of species. Ultimate, this will provide for field based SSD's. The study was placed in a permanent meadow and used 20 m^2 screen cages to confine the NTA to treated areas and prevent immigration. A suite of sampling methods was used to collect NTA following a single application of chlorpyrifos in fall. In total 12 concentrations were applied, with 2 replicates for each concentration and 4 water control units. The study was restricted to a single sample one week after application. At the moment of writing taxonomic assessments are ongoing, but for several species clear dose-effect regressions could already be obtained. At the conference we will present a compilation of the regressions obtained and use these to construct field based EC_x – derived SSD's. Using HC_5-values based on these statistical distributions the potential environmental impact of exposure will be assessed for different ecological guilds of NTA.

TH069

Evaluating Ecological Recovery in Mechanistic Effect Models for Environmental Risk Assessment

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In environmental risk assessments (ERA) for plant protection product (PPP) one possible protection goal option at the population level is recovery (EFSA 2016). This recovery option accepts "some population-level effects of a potential stressor if recovery takes place within an accepted time period". Modelling approaches are one of the options to address ecological recovery in ERA. Since ecological recovery depends on complex processes which are related to the properties of the considered species, the population, the landscape and even the ecosystem, they are viewed as powerful tools to obtain the needed information. However, general guidelines on how to address ecological recovery in ERA, particularly in population modelling, are not yet available. Additionally, the specific protection goals (SPGs) as defined in guidance documents are mainly qualitative. Nevertheless, defining robust measures that quantify recovery is a crucial prerequisite for the evaluation of risk posed by a potential stressor to populations. Herein, we suggest and exemplify an approach to quantify ecological recovery after pesticide application for population modelling studies using stochastic models (such as IBMs), in the context of ERA. The approach is based on a population's normal operating range (NOR) estimated from a set of replicate time series derived from control simulations depicting the conditions in the absence of a stressor. The NOR is compared to model simulations incorporating the impact of a stressor to detect the amplitude and duration of an effect for different endpoints, such as population size, sex ratio, or age distribution. To ensure sustained recovery the approach takes the entire annual population dynamics of the considered species into account. We exemplify this approach using an individual-based, spatially-explicit simulation model, which simulates the population dynamics of non-target species impacted by a potential stressor. This presentation will provide a basis for discussions how ecological recovery in mechanistic effect modelling for ERA can be addressed quantitatively and how recovery abilities of populations can be assessed.

TH070

A new screening approach for testing natural soil communities in the laboratory

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The current risk assessment for soil organisms is structured in different tiers. In the first tier, the impact of a substance on single species is investigated in the laboratory, whereas in the highest tiers communities can be tested in a complex field study. The former one can be controlled in the lab while realism is low, the latter one is close to realism while the methodology has to be adapted to counterbalance the high natural variability. In the project *Nanomobil* sponsored by the German Federal Ministry of Education and Research (BMBF) the effects of silver nanoparticles on the soil mesofauna are examined in terrestrial model ecosystems (TME). To set up the the long term study it was useful to get first information on the community effects in order to select an optimal concentration range for all species groups involved . Therefore a new and easy short-term screening method with natural soil communities was developed. On a natural grassland 70 soil cores (5 cm diameter) were taken close to each other and the soil cores including the natural community were placed in a special setup in two boxes in the laboratory. Five different concentrations of silver nanoparticles (AgPure) as well as four concentrations of a toxic reference ($AgNO_3$) were applied to the soil core surfaces and afterwards the soil cores were stored at 20 °C for two and four weeks respectively. After these different exposure periods, the soil organisms were extracted from the soil cores for 14 days in a McFadyen extractor according to the ISO 23611-2 guideline. In this presentation the dose response related results for oribatid mites and collembolans are shown and the usability of the screening test in the risk assessment is discussed.

TH071

Age Sensitivity Distribution of the Springtail Folsomia candida: An Aquatic Test Approach

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Age distribution is an important factor that affects the population dynamics of an organism. During exposure to chemicals, the response of different age groups can be used to potentially predict population effects in the laboratory. This technique, tagged "Age sensitivity distribution", is analogous to the common Species sensitivity distribution used in ecotoxicology to extrapolate effects from various species to community level. Although, a similar technique has been used in aquatic ecotoxicology to determine life-stage sensitivity, it is unpractical in soil ecotoxicology because of the excessive time and work demands. In view of this we employed an aquatic test approach which also simulates soil pore-water exposure. Using the age sensitivity distribution, we explored the possibilities of extrapolating effects from individuals to population for the Springtail *Folsomia candida*. This was undertaken by exposing different age groups (3, 6, 12 and 22 day old) of the test species to silver nitrate in water, and subsequently, monitoring the time course of survival, daily for 14 days. The results show an increasing trend of sensitivity with increasing age except for 22-day old animals which were more sensitive than 12-day old animals. 7 days LC50 estimated for 3, 6, 12 and 22 day old animals were 0.31, 1.23, 31.56 and 2.29 mg $AgNO_3$/L respectively. The higher sensitivity of the reproductive-active 22-day old animals was attributed to their increased energy requirements. The susceptibility of newly hatched juveniles, as well as, reproductive-active adults, suggests that population effects can be induced in the field, even at low concentrations. Furthermore, the aquatic test approach proves to be valuable to derive time dependent lethal and sub-lethal toxicity information with little use of resources. With these results, we show that age sensitivity distribution can be a relevant tool in soil ecotoxicology.

Advances in Exposure Modelling: Bridging the gap between research and application (P)

TH072

Improving industrial on-site environmental exposure assessment by adapting SimpleTreat

L. Pontal, C. Durou, CEHTRA SAS / -

An exposure assessment must be carried out either as part of the chemical safety assessment or to support exposure-based waiver for REACh registration. The exposure assessment shall cover all relevant life-cycle stages including manufacture, formulation, industrial uses and service-life. The default worst-case release factors assume emissions of chemicals to water (before STP); for example, the release factor is 6% for the manufacture of the substance and applied to daily quantity of the substance. The calculations of the exposure of environmental compartments (water and sediment, local soil) in the vicinity of a manufacturing site request first to model the distribution of the chemical in the sewage treatment plant. The model SimpletTreat 3.1 is recommended for the estimation of the fate of the substance in a biological STP (6-box or 9-box). However, the model is basically configured to model the fate in a standard municipal STP (city of 10,000 inhabitants). This configuration fits for the purposes of releases directed to municipal STP (example of consumer uses), but it may not reflect the situation of an industrial STP. The use of this default approach may lead to an overestimation of the release of substance to the environment. This is of particular concern for large-manufacturing sites. One possibility to refine such a conservative assessment is to model the fate of the substance in the industrial STP. The present work focuses on the adaptation of the recommended tool Simple Treat for an industrial EU manufacturing treating on-site the effluents though a 11-box sewage treatment plant. The poster will provide a case study including an initial conservative and improved exposure assessment, emphasis the opportunities for increasing relevance in environmental risk assessment performed for REACh.

TH073
Industrial SimpleTreat - an updated model for predicting the fate of chemicals which enter industrial wastewater systems; model validation and use in a regulatory context
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Chemicals released to the sewage system may eventually enter the aquatic ecosystem; their removal in waste water treatment plants (WWTPs) is a key step in reducing the risks to the receiving environment. Understanding the fate of chemicals in WWTPs is therefore crucial in performing a realistic risk assessment. In Europe SimpleTreat is the recommended model to estimate chemical removal for environmental risk assessment of industrial chemicals (REACH), biocides (BPD) and pharmaceuticals (EMEA). SimpleTreat is a multimedia box model of a conventional activated sludge WWTP with primary and secondary sedimentation. A recent ECETOC taskforce made up of various industry members provided input to Radboud University Nijmegen in the Netherlands to adapt SimpleTreat to better simulate the behaviour of chemical substances in industrial waste water treatment plants (WWTPs). Industrial WWTPs differ in a number of ways when compared with the municipal WWTPs the model was initially developed to simulate. A scientific publication on this project was made available in 2016, aiming to document and justify the new models use in Chemical Safety Assessments under REACH. A validation study with numerous chemicals showed fair agreement between predicted and measured concentrations collected from the literature. Further validation was also performed and published using data on pharmaceutical chemicals; however, data on the behaviour of chemicals in WWTPs is generally difficult to obtain. To gain wider acceptance within the regulatory environment, further data or collaborative projects would be of great benefit in the advancement and acceptance of this model for use in the simulation of chemical behaviour in both municipal and industrial WWTPs. In the process of updating the model, questions were raised with regards to the suitability of the currently recommended default biodegradation rate constants for model calculations. According to REACH and the TGD, the biodegradation rate constant of substances which are inherently biodegradable, but fail to fulfill certain stringent criteria, should be set to zero. This appears to cause unrealistically high release estimates for chemicals which are non-sorptive and non-volatile. An update on progress is presented with a view to furthering the discussion and investigation surrounding this area of science.

TH074
Implications of prediction of climate change impacts on the fate and transport of pollutants in multimedia environment.
Y. Lee, Seoul National University; L. Chang, D. Lee, Seoul National University / Environmental Planning Institute Graduate School of Environmental Studies
The influence of climate change on the fate and transport of pollutants (Phenanthrene, Chrysene, Benzo[a]pyrene, Benzo[g,h,i]perylene) in the environment was assessed using a region-specific multimedia model, KPOP-CC. The modeling domain covers South Korea where the spatial resolution in air is 30km X 30km with four vertical layers up to the stratosphere. Simulations were performed with 3-hour meteorological data for the period 1956-2100 under RCP 8.5 scenario. Changes in the long term average concentrations due to climate change were not drastic, but the directions of changes varied depending on the chemical. For instance, the concentration of Phe in air decreased (3%) while those of Chr, BaP and BghiP increased (up to 20%). Among the rate constants for the removal processes analyzed in this study, those for advection (kadv) was found to be largest. The magnitude of depends on the spatial resolution of models used and is independent of chemical properties. The rate constants of photodegradation (kphoto) for Phe, and those of particle-phase wet deposition (kPwet) for Chr, BaP, and BghiP were of the second largest magnitude. The magnitude of these rate constants are varied by meteorological factors modified due to climate change and the resulting changes in fate properties. Decrease of windspeed (decrease of kadv) acted to the increase atmospheric concentration of all chemicals. For Phe, the increase of kphoto due to the temperature rise exceeded the decrease of kadv, resulting in the decrease of total removal rate constant (kTotal) and the increase of the air concentration. For Chr, BaP, and BghiP, kPwet decreased due to the decrease in particle-phase fraction caused by the temperature rising, which was larger than the effect of precipitation increase. Decrease in kadv and led further to a decrease in kTotal, which eventually increased the air concentration. The results suggest that the analysis of the magnitude and variations in the rate constants of removal processes could effectively illustrate the influence of climate change on the fate and transport of pollutants as a function of the chemicals' fate properties. Moreover, depending on the spatial resolution of the model used, the effect of advection on the fate and transport of pollutants can offset or reinforce the effects of those of other processes, implying that the direction and magnitude of the predicted concentration change can be substantially influenced by the spatial resolution adopted by the model.

TH075
Influence of climate change (CC) on the multimedia distribution of volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated dibenzo dioxins/furans (PCDD/Fs)
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The objectives of this study are to quantitatively assess long term influences of climate variables on the distribution of 3 VOCs, 5 PAHs, and 8 PCDDs/Fs among the four environmental media (i.e., air, soil, water, and sediment) and how the influences vary with the fate properties of the compounds. The influences of CC were estimated by assessing the changes in the fugacity ratios (FRs) between the pairs of air and soil, air and water, water and soil, water and sediment, and soil and sediment. The FRs were calculated under the RCP8.5 scenario using a regional scale geo-referenced multimedia model (KPOP-CC) developed to simulate the fate and transport of organic compounds in the multimedia environment of Korea under various CC scenarios. KPOP-CC was evaluated to agree with observed data within one order of magnitude. The total period of simulation was 145 years (from 1956 to 2100) where CC was assumed to begin from 2006. The geometric mean of the fugacity ratios over the period from 2091 to 2100 was used for the assessment of the CC influences. Due to CC influences, FR of water to air ($FR_{water/air}$) decreases for all the VOCs (~22%) and the PAHs up to of 3 rings (~10%) while it increases for the PAHs of 4, 5, and 6 rings and PCDD/Fs (~380%) as compared to those before CC. $FR_{soil/air}$ shows marginal to modest increases for all the compounds (~143%) with PCDDs/Fs showing substantially increased ranges (~1990%). $FR_{sediment/soil}$ tends to decrease (~70%) except for the PAHs of 5 and 6 rings and 1,2,3,4,6,7,8 HpCDD. For all the compounds, $FR_{water/soil}$ tends to decrease to some extent (~78.8%) while $FR_{sediment/water}$ increases considerably (~305%). Among the climate variables, the atmospheric temperature shows the strongest correlations with the change in FRs, indicating that the change in the atmospheric temperature caused by CC may be a principal variable that modifies the multimedia distribution of organic compounds. The VOCs and the PCDD/Fs appear to be influenced by temperature more than PAHs. The CC induced change in FRs involving air (i.e., $FR_{soil/air}$ and $FR_{water/air}$) are also correlated with the rainfall intensity, suggesting a substantial change of the wet atmospheric deposition due to CC. The detailed analysis of the changes in FRs with respect to the fate properties of the compounds will further be presented.

TH076
Development of the IIAQ-CC model to assess the impacts of climate change on the indoor air quality
L. Chang, Seoul National University / Environmental Planning Institute Graduate School of Environmental Studies; Y. Lee, Seoul National University; D. Lee, Seoul National University / Environmental Planning Institute Graduate School of Environmental Studies
It is generally acknowledged that climate change (CC) will affect indoor air quality. To quantitatively predict the impacts of CC on indoor air quality, an integrated indoor air quality model under CC conditions (IIAQ-CC) was developed for organic chemicals in the vapor and the particulate phases. For assessing the CC impacts on indoor air quality, the model takes into account the changes due to CC in emission, fate and transport of pollutants in indoor air, natural ventilation rate as a function of wind speed, difference in indoor and outdoor temperature, and frequency of window opening. To evaluate the model, measured data of formaldehyde was used for the periods of 2012/12-2013/02 and 2014/12-2015/02 (n=103, 24 households). The slope of the concentration change with respect to the indoor-outdoor temperature difference was compared between measured (-3.9%/ºC) and simulated (-4.4%/ºC) to find no significant difference ($p< 0.05$). As a case study, simulations of formaldehyde carried in ventilation air and from indoor sources of vinyl flooring were conducted for the periods of 2011-2040 (F1), 2041-2070 (F2), 2071-2100 (F3) under the RCP8.5 scenario and the results were compared to those under no CC scenario in the environment of Seoul. Due to CC, the annual average indoor concentrations of formaldehyde for the F3 period increase by 7% (t-test, $p< 0.05$). The maximum annual average concentration for the F3 period increases by up to 24% in indoor. Change in the average concentration of formaldehyde in indoor air for the period from F1 to F3 is greater than that in outdoor (paired t-test, $p< 0.05$). These results suggest that indoor air pollution with formaldehyde increases faster than that of outdoor under the CC conditions.

TH077
A Double-Diffusion Model to Quantify the Adsorption Effects of Indoor Surfaces on the Exposure to Chemicals Encapsulated in Products
L. Huang, University of Michigan / Dept of Environmental Health Sciences; O. Jolliet, University of Michigan
Chemicals encapsulated in products can constitute a major emission source in the indoor environment. Modeling methods have been developed to predict the release of these chemicals into indoor air, but earlier models generally consider ventilation as the only loss process of the chemicals in indoor air. Recently, increasing attention has been paid to the adsorption effects of indoor surfaces as a loss process for indoor chemicals, especially for semi-volatile organic compounds (SVOCs). Previous studies have used simple assumptions to model the adsorption effects which is likely not the case in reality. This study thus aims to better simulate the

adsorption effects using a more sophisticated model to help elucidate the role of the adsorption effects on the release of chemicals and near-field human exposures. A double-diffusion model was developed which describes the diffusive emissions of chemicals from materials, the subsequent loss by ventilation, and the loss by diffusive adsorption by indoor surfaces. A typical North American household was modeled. The target chemicals were assumed to originate from a 3mm-thick vinyl flooring, and the indoor surfaces considered were gypsum walls and ceilings. The model system was solved numerically using Method of Lines discretization with a time step of 1 min. The model was tested for three chemicals, a typical VOC (ethylbenzene), a typical SVOC (DEHP) and one in-between (tetradecane). Results show that adsorption on walls and ceilings can significantly lower the peak air concentration of chemicals emitted from flooring, from a factor of 2 for ethylbenzene and a factor of 6 for DEHP. The predicted air concentration converges with that predicted by a single-diffusion model (not accounting for adsorption) after a short period for ethylbenzene and tetradecane, but the factor of six reduction in exposure remains for DEHP even after 15 years. Adsorption also increases the chemical mass released from flooring by a factor 7 for DEHP. Overall, this study shows that for VOCs the adsorption effect is negligible, while it is crucial to consider for SVOCs for which indoor surfaces can indeed be viewed as infinite sinks in the time scale of consumer use. Future research is needed on chemicals in between VOCs and SVOCs, for which the adsorption effect is non-trivial, the indoor surfaces are finite sinks and may serve as secondary sources.

TH078

Environmental exposure scenario for reagents used in-vitro diagnostics

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In-vitro diagnostic assays contain a range of different substances that may be released to the environment via a sewage treatment plant, if the effluent from diagnostic instruments is discharged into the sewer system. Components of *in-vitro* diagnostic assays have so far not been subject to an environmental risk assessment under regulatory frameworks. Therefore, exposure scenarios have not yet been developed. Release of liquid waste into the public sewer system is regulated at a national or often even at a local level. Therefore, it has to be assessed for each site, whether release of the effluent from *in-vitro* diagnostic equipment is compliant with local regulations including permits issued by the local authorities. Exposure scenarios were – as examples – developed based on the conditions encountered at typical use sites in Switzerland and Germany with options to adapt to other local settings. Analytical results of liquid waste generated by diagnostic equipment (Stark-Rogel *et al*. 2016) together with typical operational parameters for *in-vitro* diagnostic instruments were defined as default input parameters that can be adapted to local values. Regulatory threshold values for release into the sewage system and predicted no-effect concentrations (PNEC) for freshwater were implemented in the tool. Comparison of concentrations in liquid waste and predicted environmental concentrations (PEC) with these thresholds shall facilitate the assessment a) whether release into the sewage system is allowed and b) whether such release results in potential risks to surface water. Our model is a simplified screening assessment, not taking removal processes in the sewage treatment plant, such as biodegradation or adsorption into account. It identifies potentially critical substances for more advanced assessment. Two example assessments of fictitious, but typical hospitals in Germany and Switzerland mainly using default parameters with some specific adaptations indicated the few substances that cause potential concern. Assessment of site-specific waste volumes, or site-specific determination of substance concentrations could be performed to refine this model. Furthermore, assessment with more advanced exposure tools could be performed for the identified substances of concern taking removal processes in the sewage treatment plant into account. **Reference** V. Stark-Rogel, R. Borner and J. Backmann, Mitt Umweltchem Ökotox, 2016, 2, p. 44-50

TH079

Integrated external and internal exposure to chemicals: the INTEGRA computational platform

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The objective of INTEGRA project (CEFIC LRI) was to bring together all available information within a coherent methodological framework for assessing the source-to-dose continuum for the entire life cycle of substances covering an extensive chemical space. Hence, the major output of INTEGRA is a computational platform that integrates environmental fate, exposure and internal dose dynamically in time, including also inverse modeling for exposure reconstruction and HBM data assimilation. Within the frame of INTEGRA, (Bis(2-ethylhexyl)phthalate) DEHP was chosen as a critical compound related to consumer exposure, due to the increased scientific and regulatory interest of the latest years. The aim of the study was the identification of potential exposure scenarios (or combinations) among intended uses of DEHP and how these are translated into internal exposure, as well as they are reflected into biomonitoring data. Indented uses of DEHP include building and construction materials, car interior, clothing, food packaging, children's products, gloves, medical devices, PCPs and cosmetics. Scenarios related to the overall cycle assessment of DEHP in building materials seems to be of excessive interest, DEHP in vinil flooring being dominant among them. The overall life cycle assessment of vinyl flooring manufacturing showed that environmental contribution to overall aggregate exposure to DEHP is negligible (either to ambient air contamination or due to food transfer). Thus, aggregate exposure to DEHP is attributed to the pathways involved due to vinyl flooring emissions. Under a typical scenario in a common residential dwelling (surface area of 270 m^2 and air exchange rate of 0.5 h/r) characterized by DEHP gaseous emissions of 200 μg/h (vinyl flooring and other plastic materials), the concentrations of DEHP in the gaseous, particles and dust phase are equal to 1.5 μg/m^3, 21 μg/m^3 and 4400 μg/g settled dust. Overall daily intake varies between 0.2 to 10 μg/kg-bw, depending on the exposure scenarios considered. The latter are age-dependent: adults are exposed mostly through inhalation and infants through non-dietary ingestion. For a common repeated aggregate exposure scenario of 2 μg/kg-bw, the DEHP internal dose in venous blood and in adipose tissue (where bioaccumulation is clearly observed) reach a quasi-state equilibrium of 0.07 and 0.4 μg/L respectively. The expected urinary concentrations of MEHP, 5-OH MEHP and 5oxo-MEHP are 3.1, 16 and 8 μg/gCr respectively.

TH080

Regional scale modelling of PCBs from a highly contaminated site in Northern Italy

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Brescia is a city located in Northern Italy which suffers the impact of its large scale industrial development on the surrounding environment. This development started at the beginning of 1900 when different industries such as foundry, steel, mechanical, weapon and chemical grew up. Among chemical industries, Caffaro S.p.a. produced PCBs for about 50 years (1930-1984) and its surrounding areas were found to be heavily contaminated with high concentrations in soil at mg/kg levels. For this reason this area was declared National Relevance Site (SIN) for remediation by the Italian authorities. The aim of the present study was to investigate the potential of the contaminated area in driving the PCB contamination at regional scale up to about 100 km from the point source and the current effects on air concentrations. Different sampling campaigns were organized to collect samples of soil and leaves along four 100 km transects (one sampling point every 7 km) that ran in NW, NE, SW and SE directions considering the production plant as starting point. Woods soils were chosen to avoid to collect samples altered by tillage activities. Leaves were sampled above the corresponding soil sample. In each sample the following PCB congeners were determined: PCB 28, PCB 52, PCB 101, PCB 153, PCB 138, PCB 180 and PCB 209. PCB 209 was included because it can be considered a marker of Caffaro contamination, the only world producer of Fenclor DK, a technical grade decachlorobiphenyl mixture. The results were analyzed to understand the presence of a spatial gradient of decreasing pollutant concentration with distance from the source. Fingerprint data were used to run a number of simulations with a dynamic air-vegetation-litter-soil model in order to 1) predict the order of magnitude of fluxes at each point considering the actual soil and leaf concentrations, 2) understand the source strength in order to predict a temporal emission profile from the site, 3) evaluate the importance of other sources and processes involved in the contamination at a regional scale.

TH081

Chemical Preservation of Semi-volatile PAH Compounds at Ambient Temperature: A Sediment Sample Holding Time Study

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Site investigations require the collection and analysis of representative environmental samples to delineate impacts, risk, and remediation options. When samples are removed from the environment, concentrations of semi-volatile polycyclic aromatic hydrocarbons (PAHs) begin to change due to various processes such as evaporation, adsorption, photo and microbial degradation. Preservation techniques are used to minimize these changes between collection and analysis. The most common techniques are refrigeration, freezing, and acidification. Regulatory agencies have developed holding time requirements of 14 days for PAH in soil/sediment samples stored at < 6°C. The technical basis for these requirements is not well defined yet failing to meet these criteria may deem the analytical results not valid. This study examined the effectiveness of using chemical preservatives for sediment PAH samples to defensibly extend the prescribed holding time requirements in the absence of refrigeration. Sediment samples collected at three (3) separate sites impacted with petrogenic and pyrogenic PAHs were analyzed as preserved (sodium azide) and unpreserved samples at defined time intervals up to 60 days. Statistical analysis of the data demonstrated no degradation of PAHs in the sediment samples preserved with sodium azide for up to 60 days at ambient or 4°C. Sodium azide preservation provides a useful technique to ensure sediment sample integrity in situations where maintaining holding temperature below 6°C during transport to the laboratory may be uncertain, e.g., during sample collection in remote locations.

TH082

Risk assessment performed by using multiple lines of evidence: a case study with soil gas and flux chamber measures

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The collection and use of soil gas data and vapor flux measures from the ground using flux chambers are growing techniques in Italy. The aim of collecting such data is to better understand whether there is an actual human health risk generated by the volatilization pathway, for contaminants in soil, groundwater and non-aqueous phase liquids, in the indoor and outdoor environment. These approaches are validated by specific regulation and technical guidelines in Italy. In cases where indoor/outdoor inhalation exposure pathways have been identified as a concern, it has emerged that the standard human health risk assessment procedure used in Italy often provides conservative outputs, especially regarding indoor air exposure. This is particularly the case for petroleum hydrocarbons, where the actual concentrations in air at the point of exposure have been found to be much lower than those predicted by the simulation model first developed by Johnson and Ettinger, and used in Italy. A field case study to assess the success of a simulation model in predicting the contribution of contaminated vapors originating from soil and groundwater contamination versus other sources of contaminants in air, for a petrol station, will be described. The applied method combines multiple lines of evidence. At this petrol station, petroleum hydrocarbon contamination had been found in groundwater and soil. First, the conceptual model of the site was defined. Second, forward risks and threshold target remediation limits were calculated in a classical way – relying on a simulation model to predict the migration of contaminants from solid/liquid matrix to the gas matrix – which indicated unacceptable risks may be present in comparison to legally-defined limits. Thirdly, a more detailed investigation was undertaken to measure the actual transfer of the contamination from the solid/liquid matrix to the gas matrix and to estimate the degree of biodegradation of the contamination from ground/groundwater to the measurement/exposure point above the ground. Finally, the predicted concentrations in indoor and outdoor air were calculated using an integrated approach (combining the empirical data with modelling) and through use of attenuation coefficients. The results of this study demonstrate that the collection of targeted empirical data allowed a more refined understanding of the actual risks to human health and development of site specific threshold target remediation limits.

TH083

Fertilizers Environmental Exposure Tool: Advances in environmental exposure assessment of micronutrients in fertilizers

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In order to comply with the requirements of REACH (Regulation (EC) No 1907/2006), it is necessary to perform a quantitative exposure and risk assessment for all hazardous substances manufactured and imported above 10 t/year. Since the existing local scale environmental exposure tools (ECETOC TRA, Chesar, EUSES) do not take into account direct emissions to the soil compartment and direct and indirect releases from treated agricultural fields to surface water, these tools are not suited to cover environmental exposure assessment of e.g. fertilizer uses. Furthermore, important output pathways for fertilizers via crop uptake and harvest are generally not considered in these tools. Therefore, to improve exposure and risk assessment of potentially hazardous micronutrient compounds, such as e.g. Cu and Zn, a spreadsheet-based fertilizers environmental exposure tool has been developed by ARCHE and Fertilizer Europe members. This tool can be used to calculate local exposure of metallic micronutrients for soil, sediment and surface waters. Conceptually, the tool has been based upon existing REACH exposure modelling, but adapted for fertilizer uses by adopting relevant information from fate models from other chemical legislations to allow the inclusion of important processes such as drift and runoff and taking into account the good agricultural practice to apply fertilizers according to crop needs and only into soil with low micronutrient availability. The resulting tool is more representative for fertilizer uses, compared to the industrial or municipal local settings implemented in the standard REACH models. In addition, crop-specific data were gathered to determine the portion of micronutrient taken up by the crop and exported from the field with the harvest. Fertilizer uses with similar use conditions were grouped and for each group, a specific environmental release category (spERC) with realistic worst-case estimates for emissions to the environment was determined. The tool can be used to calculate local predicted environmental concentration (PEC) for soil, sediment and surface water. These PEC's can be compared in the tool with predicted no effect concentrations (PNEC's) from the corresponding REACH dossiers, to predict under which conditions the micronutrient compounds are safe to use. This tool will help harmonization and communication of micronutrient environmental exposure and risk assessments within the supply chain.

TH084

Mathematical model for radionuclide transport affected by rate-limited sorption

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Experimental and theoretical studies have been undertaken to understand the transport of radionuclides in subsurface environments because that the radionuclide transport in groundwater is one of the main pathway in exposure scenarios for the intake of radionuclides. Mathematical model for multiple nuclides transport equations coupled with first-order sequential decay reactions can serve as fast and cost-effective tools for predicting the transport of the predecessor and successor species of radionuclide decay chain. However, only few mathematical model that were solved for coupled multiple radionuclides transport equations are available in the literature. For mathematical convenience, most models currently used to simulate radionuclide decay chain transport assume instantaneous equilibrium between contaminant in the dissolved and sorbed phases. Research has demonstrated that rate-limited sorption can have a profound effect upon the solute transport in the subsurface environment. By making the equilibrium sorption assumption, the potential effects of rate-limited sorption/desorption are not considered and cannot be examined. In this study, we develop an analytical model for radionuclide decay chain transport subject to rate-limited sorption. The derived model is applied to investigate the effects of the rate-limited sorption on plume migration of radionuclide decay chain.

TH085

How Advances in Exposure Modelling can technically become included in Regulatory Context

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The implementation of novel results in environmental models which are used in regulatory context is a necessary step of scientific development. However, the implementation process must fit in the strict framework of regulatory modelling and related version control. Regulatory accepted models are being developed by independent research groups. Each model has a "general/research" version as well as a regulatory version. The general versions offer the highest flexibility, and contain latest developments in modelling. Model changes are usually incorporated in parallel to the research progress of their authors. The regulatory versions of the models offer less flexibility: While substance and application related parameters can be freely chosen by the user, modification of scenarios is either not possible or not straightforward. Regulatory models undergo a strict version control, which is organized by FOCUS DG SANTE (FOrum for Co-ordination of pesticide fate models and their Use). The group controls the models from the scientific and technical point of view. Latest developments shall be included in the models after discussing them in the version control group. New model versions can be posted to the FOCUS DG SANTE homepage (http://eusoils.jrc.ec.europa.eu/projects/focus-dg-sante) only after detailed technical testing. This procedure ensures that models reflect scientific development, but the most important features in regulatory modelling are conserved. The relevance of new processes must be discussed before their implementation in regulatory models. Moreover, latest developments have to achieve a certain maturity before they can be implemented in regulatory models: In contrary to confirmed and scientifically accepted results, new ideas and hypotheses might be still proven as false. Accordingly, following criteria can be proposed for the implementation of novel processes in regulatory models: General acceptance: More independent research groups shall work on the topic. Related publications have to be independently cited and and must go beyond conference proceedings. Fit in regulatory framework: Processes must be discussed during development of guidance documents before their straight implementation in new model versions Version control: current good practice of version control shall be continued in the future. Accepting the above proposed criteria as a practical guidance would ensure sustainable and smooth development in future regulatory modelling.

TH086

Influence of uncertainty on decision-making for reduction of pharmaceuticals in rivers

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In the last years, increasing interest has gone into chemical exposure models (CEM) for the assessment of chemical concentrations throughout Wastewater Treatment Plants (WWTPs) and into rivers to assist decision-making. In addition to common challenges faced by any ambitious modelling exercise (balance between realism and simplicity), microcontaminant modelling poses particular problems related to the multiple potential (bio-)chemical behaviours, and the relative paucity of available data to feed the models. This implies high uncertainty in predicted environmental concentrations (PECs), which should be taken into account when evaluating measures to reduce microcontaminants loads. Even though a few studies have incorporated uncertainty around model development, the influence of that uncertainty on selecting potential management measures has never been reported. To fill this gap, we developed a CEM which describes the fate and removal of pharmaceuticals along WWTPs and the river network. The model has 3 key parameters: human pharmaceutical consumption and excretion including losses over the sewerage (F), and pharmaceutical degradation constant in WWTPs (k_{WWTP}) and rivers (k_{river}). The Llobregat river basin (NE Spain) was selected as the case study and diclofenac as the target compound. We calibrated the model using a Bayesian approach, and subsequently applied scenarios that mimic more advanced wastewater treatment by varying the values of k_{WWTP}. Scenarios of different degree of uncertainty were also generated by varying the dispersion of the probability distributions of model parameters to assess the impact of uncertainty on decision-making. We found that the calibrated CEM can accurately predict measured diclofenac loads at 9 locations along the Llobregat river and at the influent and effluent of 2 WWTPs ($R^2 = 0.95$). We also identified that the installation of tertiary treatments results in apparent reductions in diclofenac concentrations irrespective of the scenario of uncertainty evaluated. Nevertheless, upgrades in secondary treatment results in apparent reductions, but they were dependent on the degree of uncertainty. We conclude that prediction uncertainty greatly influences the decisions that River Basin authorities must make in order to reduce the loads of diclofenac released by WWTPs into rivers, so measures to quantify and reduce prediction uncertainty should be also a management priority.

TH087
SETAC Exposure Modeling Interest Group
T. Gouin, Unilever / Safety and Environmental Assurance Centre

Measuring and Estimating Dose Metrics: Linking Exposures to Effects for improved Chemical Risk Assessment (P)

TH088
Relating in vitro to in vivo: bioavailability an essential element
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In vitro assays are often applied for unraveling toxicological mechanisms of action. This is a major strength of these assays and will also lead to a reduction in animal in vivo testing. Such mechanistic studies are mostly qualitative. To extend the use of these assays to hazard characterization and perform quantitative in vitro to in vivo extrapolations (QIVIVE), the dose in the vitro test should be linked to a dose in a whole animal study. The freely dissolved concentration is one example of a dose metric that may link in vitro (cell medium) to in vivo (blood). In order to be successful, one needs accurate information about the dose in the in vitro test. Nominal concentrations may be appropriate for “simple compounds”, but for more hydrophobic or volatile chemicals, information about the actual dose is needed. Exposure modeling is very useful in identifying these more difficult compounds and can also be applied to estimate the actual bioavailable dose. This presentation will focus on the strengths, but also some of the limitations, of the concept of the freely dissolved concentration. Examples from some of the earlier publications as well as new experimental data for cationic surfactants will be presented to identify both the advantages and potential limitations in estimation and measuring the actual dose and bioavailability in in vitro studies. New data on the dose of a series of benzalkonium chlorides in a cell assay with a fish gill cell line for example show that deviations of measured concentrations from nominal concentrations in the test medium depend, as expected, on the carbon chain length. However, additional factors that influence these deviations are the exposure time as well as the exposure concentration. Earlier studies have also shown that the technique applied to spike a compound in the medium may affect the outcome of the test. Other dosing systems, based on passive dosing from a sink, may circumvent these confounding factors but such dosing systems are available for only specific types of compounds and it is not feasible to apply those techniques in high throughput screening. Also the hypothesis that the freely dissolved concentration represents the bioavailable and active form is not always correct, in particular in non-equilibrium situations, and this will be illustrated with a few examples. More general strengths, limitations and research recommendations of in vitro-in vivo extrapolations will be discussed.

TH089
Using predicted and measured physicochemical properties for better planning and interpretation of toxicity tests
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Planning and interpretation of *in vitro* tests can be significantly improved by considering the physicochemical (PC) properties of the tested chemicals. Therefore, we suggest dosing the chemicals dependent on their solubility, baseline toxicity and speciation into a bioassay, instead of dosing all chemicals at the same concentration level. The solubility of a given test chemical should always be taken into account, since it cannot only vary substantially between different chemicals, but also for a single chemical depending on the composition of the assay medium. Hydrophobic organic chemicals typically poorly dissolve in pure water or physiological saline, but the solubility in cell culture media can be orders of magnitude higher due to binding to proteins and lipids. Baseline toxicity should also be considered, as it might either be the endpoint of interest of the bioassay or interfere with the quantification of a specific endpoint. The tendency of a chemical to partition to membrane lipids has been shown to correlate with baseline toxicity and can thus be used to predict non-specific toxicity. The pK_a values for the test chemicals have to be checked, because the PC properties can differ substantially between the neutral and ionized species of a chemical, often leading to a pH dependence of toxicity. Knowledge of PC properties does not only support the planning of new experiments, but also helps to identify chemicals that are outside the applicability domain of an assay. This includes volatile chemicals in open test systems, hydrophobic chemicals in purely aqueous media, chemicals that show fast degradation/hydrolysis and permanently charged chemicals exhibiting slow diffusion through biological membranes that might not be taken up by cells or test organisms within the timeframe of a bioassay. PC properties can be acquired by own experiments, by collecting experimental literature data, or by using computational prediction tools. Measuring PC properties for all chemicals of interest in-house would be ideal, but is unrealistic in most lab routines. Researching previously determined PC properties from the literature is a more convenient way, but can also be time consuming and often no data are available, especially for newly emerging pollutants. A useful alternative to measuring PC properties are computational tools, which allow PC predictions for a large number of chemicals within a short time, but can be prone to high uncertainty, which has to be considered.

TH090
Expanding the evaluation of the chemical activity hypothesis for toxicity assessment
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Regulations are being applied to evaluate the potential hazard, exposure and risk of chemicals to ecological receptors and humans. Evaluating the potential hazard and risk for all chemicals using traditional monitoring and animal testing data is not feasible. Consequently, there is value in maximizing the applicability of existing measured data for toxicity and risk assessment through the development and evaluation of models and Quantitative Structure-Activity Relationships (QSARs). The chemical activity hypothesis (model) has shown promise for characterizing toxic effects for acute lethal baseline narcosis for certain chemicals and test organisms. The hypothesis has been evaluated for a relatively limited range of species and taxa and the focus has been on the acute lethal endpoint and neutral organic chemicals. This project seeks to expand the evaluation of the chemical activity hypothesis to a broader range of organisms, effect endpoints and chemicals. A large aquatic ecotoxicity database has been compiled from publicly available sources and the peer-reviewed literature (ca. 1949 to 2012). The initial database includes more than 100,000 fish toxicity test entries for approximately 2,500 organic chemicals with molar mass ranging from 27 to 1356 g/mol and octanol-water partition coefficients (log K_{OW}) ranging from -7.3 to 11.4. Screening-level data quality assessment methods were developed and applied using a 1-compartment toxicokinetic model and chemical properties to identify test results considered to be either “unreliable” or “conditionally acceptable”. The general approach of the data quality screening is to identify key sources of possible experimental error realizing that it is impossible to identify and address all possible sources of experimental uncertainty in the database. Guidance for selecting chemical properties for chemical activity calculations is also presented. Relationships between chemical activity and predicted mode-of-action classifications are examined. A subset of well documented toxicity tests is used as a case study of “acceptable quality” data to examine the applicability of the activity hypothesis.

TH091
Measuring aquatic mixture toxicity by the direct application of equilibrium passive samplers in passive dosing mode
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Environmental mixture toxicity results from exposure to undefined organic pollutant mixtures. The application of toxicity bioassays to measure this mixture toxicty is challenged by difficulties in sampling the complete contaminant profile at the correct bioavailable levels. Furthermore, it is difficult to properly reproduce and maintain these sampled mixtures in the test. Here, passive sampling and dosing can play a role. For sampling environmental mixtures, passive sampling accumulates all compounds and targets their dissolved concentrations. If the passive sampling polymer is subsequently used in passive dosing mode, it is possible to translate the sampled mixture back into the dissolved concentrations originally present in the field and to keep these constant. This study applied equilbrium passive sampling to first sample aquatic contaminant mixtures in a WWTP and the receiving river, and

then used these in passive dosing mode in the Microtox® and ER Calux® bioassays for determining the mixture toxicty. In parallel, mixture toxicity was determined using the traditional sampler extraction and spiking approach. The latter resulted in a higher measured mixture toxicity compared to passive sampling and dosing, likely due to up-concentration of the contaminants in the sampling polymer offsetting any lack of buffering against test losses. To investigate this in more detail, mixture concentration-response testing was performed. For the WWTP inflow samples, sampler extracts had to be diluted by a factor of over 500 before no toxicity was measured in the Microtox test. For the WWTP outflow and river samples this was achieved by around a factor 10 dilution. However, a detailed interpretation is difficult due to the skewed mixture profile accumulated in the passive sampler and which is directly reproduced in the toxicity test with extraction and spiking. In contrast, with the passive sampling and dosing approach a clear trend with mixture concentration was observable for the WWTP inflow samples. This is because loading the equilibrium passive sampler extract onto increased or decreased amounts of clean polymer changes the polymer concentrations of each compound by the same amount and thus the mixture profile is preserved when passive dosing. Equilibrium passive sampling and dosing can be be implemented in toxicity testing for sampling environmental mixtures at their bioavailable levels, and then reproducing and maintaining these in toxicity tests.

TH092
Exposure and Risk Assessment for Agricultural Applicator to Insecticide Chlorantraniliprole during Rice Cultivation in Paddy Field using Whole Body Dosimetry
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Chlorantraniliprole belongs to diamide insecticide that has been used to control a wide range of insects in fruit and vegetables. Assessment for agricultural applicator's dermal and inhalation exposure to chlorantraniliprole during cultivation of rice in paddy field was carried out. For dermal exposure measurement, whole body dosimetry (WBD) was performed, which consists of cotton/polyester outer clothes and cotton inner clothes. Hand exposure was measured by washing of nitrile gloves and hands, while head exposure was monitored by face/neck wipe technique. Inhalation exposure was evaluated with personal air sampling pumps and IOM sampler (glass fiber filter). Analytical limit of quantitation was 5.0 ng/mL with good linearity ($R^2 > 0.99$) of calibration curve. Recovery (82~118%) of insecticide from various exposure matrices were reasonable including field recovery (88~106%). Field exposure experiments were carried out by 10 replicates. During application, total dermal exposure was 36628.9 µg, while that of mixing/loading case was 599.1 µg. Hand exposure of chlorantraniliprole (520.4 µg; 86.9% of total dermal exposure) in mixing/loading was higher than the case of application (77.0 µg; 0.2% of total dermal exposure). Exposure of thigh and shin was high (50.8%) in case of application. Penetration rate of insecticide between outer and inner dosimeter was about 10% (upper body) and 40% (lower body). Inhalation exposure during mixing/loading (7.2 µg) was about 5 times more than that of application case (1.9 µg). Margin of safety (MOS) was calculated to be 19.5 using 75 percentile of inner cloth exposure amount, 70 kg of body weight and 360 µg/kg/day of acceptable operator exposure level, suggesting that health risk of agricultural applicator during treatment of chlorantraniliprole for rice in paddy field would be minimum.

TH093
Various approaches for equilibrium passive sampling of POPs in lean and lipid rich fish tissue
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Equilibrating passive samplers with habitat water, sediment and fish can be applied to estimate the degree of thermodynamic equilibrium for POPs between these media. which is important for understanding the process of bioaccumulation. The published method for in tissue passive sampling applies mainly to lipid rich fish tissues. For lean tissue the uptake capacity is much smaller and equilibrium is genrarally not obtained within an exposure period where fish tissue does not substantially decays. In this work we investigated various alternative approaches to obtain equilibrium faster including thinner samplers and different exposure systems/procedures. Furthermore PRCs were dosed to the samplers to monitor the exchange rate from their release. The setup allows to evaluate equilibrium and can confirm absence of depletion. For the experiments three species of fish were used: lean fish, i.e. pike-perch (Sander lucioperca), medium lipid-rich fish such as carp (Cyprinus carpio), and fatty fish, i.e. salmon (Salmo salar), containing 1%, 5% and 15% lipid respectively. Samplers were exposed to fish tissue in various forms [1] static contact of samplers with fish tissue relocating the sampler at set times, [2] rolling samplers together with fish cubes (1-2 cm) and [3] exposure of a sampler to homogenate with mixing and replacing. Samplers, fish tissue was analyzed for concentrations of PCBs, PRCs, the DDT family and, hexa- and penta-chlorobenzene. The release of performance reference compounds (PRCs) dosed to the sampler prior to exposure provides information on the degree of equilibrium and or depletion and could also be used to correct for incomplete exchange. The various methods are compared in their rate of equilibrium in relation to the lipid content as well as their final concentrations. Additionally the lipid based concentrations derived from passive sampling are compared with those obtained by classical analyses of fish tissue. **Acknowledgements:** This work was supported by the Czech Science Foundation grant No. GACR 15-16512S „Investigation of accumulation of persistent bioaccumulative toxic organic substances into aquatic organisms

Organic micropollutants in the environment: analytical challenges and engineering innovations (P2)

TH094
Identification of emerging contaminants from a waste water influenced River using high resolution accurate mass LC/MS, chemometrics and statistical analysis
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Historically pristine sources of water are increasingly threatened by contamination from a plethora of point and non-point discharges. With over 15,000 new chemicals introduced into commerce daily it is impractical to monitor each one. Non-targeted methods using HRMS can identify many compounds, but the contents of these discharges are not often available in databases. Determination of what components are actually significant and identifying them is critical, but difficult given the variability of environmental samples across time and season. This work examines LC/HRMS data of a river influenced by agriculture, hydraulic fracturing and domestic wastewater from January to November 2016 using multivariate statistics to determine compounds that are consistently being discharged into the environment and should be identified and monitored. Replicate samples of wastewater effluent, downstream, tributaries, field blanks and lab blanks were analyzed. Using chemometrics and statistical tests, the number of relevant features in the samples collected downstream of a wastewater plant as compared to at the effluent was identified as ~110 (down from >700 unique features identified in the downstream sample). Further, Non-targeted analysis using a custom database (1200 compounds with MS/MS spectra) gave positive identification of carbamazepine, diphenhydramine, enrofloxacin, fexofenadine and others in the downstream samples. These would be some of the statistically significant compounds that monitoring studies should be focused on. The non-targeted database analysis also demonstrates that although compounds of interest can be determined, there are many more compounds that cannot using a limited database. With the locations sampled across several months, statistical techniques are used to distinguish unknowns not in a database that appear consistently and thus should be identified to determine their importance as an emerging contaminant. QA/QC spikes were used to assure the appropriate use of both data processing features and statistical methods.

TH095
Micro-pollutants along the river Rhine investigated by high-resolution mass spectrometric approaches
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Over the last decades, the occurrence and fate of trace organic contaminants in water became a major environmental and public health concern. While targeted analysis allows only the detection of a limited set of compounds for which a standard is available, non-target analysis allows detecting a much broader set of compounds (including unknowns). However, data processing remains still a challenging and time-consuming task, since typically re-analysis to obtain MS/MS information is required after data analysis and selection of relevant compounds. Therefore, this study introduces the application of a high-resolution mass spectrometric screening approach by all-ions MS/MS for the analysis and identification of micro-pollutants in the river Rhine. Samples were collected from multiple locations along the river Rhine. These samples were analyzed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS). In this study, the acquisition of MS/MS spectra at varied collision energies (CE = 0, 20 and 40 eV) was performed without isolating any precursor ion (all-ion approach). The results required automated data processing based on deconvolution and information from a compound library. In a first phase, the algorithm looks for a potential match for the measured accurate masses on the low-energy channel (CE = 0 eV) with exact masses from the library. In a second phase, for each compound with a positive match at 0 eV, the algorithm considers its product ions from the library and searches for potential matches in the measured accurate masses on the high-energy channels (20 eV and 40 eV). In a last phase, the algorithm assesses the co-elution of the molecular ions on the 0 eV channel with the product ions on the 20 and 40 eV

channels. Finally, the analyst gets a summary table containing the list of compounds identified with the mass error (in ppm) and the number of product ions found. This approach allowed the fast identification of more than 60 compounds in the river Rhine River. Among these compounds a large fraction comes from active ingredients of pharmaceuticals, including antibiotics, antidepressants and heart medications. Moreover, several other compounds such as flame retardants, pesticides and their metabolites were also detected. Overall, these compounds showed a similar trend with increasing concentrations from the most upstream to the most downstream sampling locations. This clearly reflects the increased input of increasing population equivalents along the river.

TH096

JOINT NORDIC SCREENING OF EMERGENT POLLUTANTS - EFFICIENT TOOL FOR DEVELOPING MONITORING AND REGULATION

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Nordic national environmental monitoring schemes have been supplemented by a joint and coordinated screening programme since 2001. The aim of the Joint Nordic Screening of Emerging Pollutants is to obtain a snapshot of the occurrence of potentially hazardous substances in the environment both in regions most likely to be polluted as well as in some pristine environments. The focus is on little known, anthropogenic substances and their derivatives, which are either used in high volumes or are likely to be persistent and hazardous to humans and other organisms. If substances that have been screened are found in significant amounts this may result in further investiga-tions or monitoring on national level. Screening is thus the first necessary step in the consid-eration to regulate a substance. Substance groups reported in last ten years include musks, perfluorinated compounds, si-loxanes, selected biocides, phenolic substances, new brominated flame retardants, plasti-cizers and sweeteners and quaternary ammonium compounds. Next screening campaign will be performed using non-target analyses to get a more wide scope of anthropogenic pollutants. The results from screening can be used when analysing possible environmental effects of the selected substances, and to assess whether they pose a risk to the environ-ment or not. The data from these Nordic screenings have been used in the evaluation pro-cesses within Arctic Monitoring and Assessment Programme, Stockholm Convention on Persistent Organic Pollutants and EU REACH. The Nordic screening project is run by a steering group with representatives from the na-tional environmental research institutes. In addition to the screening campaigns, the group has arranged seminars/workshops related to common screening interests. The project has been financed and supported by the Nordic Council of Ministers. \n Screening reports (TemaNord), presen-tations, litterature surveys and information is found on web: nordicscreening.org.

TH097

Discovery of Novel Fluorinated and Chlorinated Contaminants in Wildlife by Non-Target HPLC-Orbitrap with In-Source Fragmentation Flagging

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The presence of unknown total organic fluorine in human and environmental samples prompted the development of a new analytical method capable of detecting and characterizing novel perfluoroalkyl or polyfluoroalkyl substances (PFASs). Using parallel full-scan and in-source fragmentation flagging functions in an Orbitrap Elite™ mass spectrometer, we previously reported a sensitive untargeted method[1] and workflow capable of detecting and characterizing novel PFASs in water by accurate mass and fragmentation flagging for perfluorinated fragment ions (e.g. $C_2F_5^-$, $C_3F_7^-$, $C_3F_5^-$ etc.). Serendipitously, the method was also capable of discovering novel organochlorine compounds by fragmentation flagging for Cl- (m/z 35 and 37) ions. Here we describe successful application of this method to wildlife samples, including to fish livers from specimens collected in China (Tangxun Lake, Wuhan City and Yangtze River at Changshu) and to polar bear serum (Beaufort Sea and the Western Hudson Bay populations). Liver and serum were concentrated using solid phase extraction and stir bar sorptive extraction methods, respectively. Between the two sites, 10 new classes of PFASs were detected in the fish livers, most of them having long fluorinated carbon chains (i.e. $C_nF_{2n+1}R$, where n>7). In polar bear serum, 4 groups of new PFASs (which were also found in fish livers) and 5 groups of new PCB metabolites were discovered. These newly detected compounds may have important toxicological relevance, and can help to explain the unknown mass balance of organofluorine in environmental samples.

TH098

Estimating Organophosphate Ester (OPE) Transport, Fate and Emissions in Toronto, Canada using the Multimedia Urban Model (MUM)

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OPEs are a group of chemicals found at relatively high levels as environmental contaminants. The usage of OPEs has increased precipitously in recent years following the listing of penta- and octa- BDEs as POPs under the Stockholm Convention. In this study we used the Multimedia Urban Model of Diamond and co-workers to estimate the transport, fate and emissions of three chlorinated (Cl-OPEs) and three non-chlorinated OPEs (non-Cl-OPEs) in Toronto, Canada. This model has been evaluated for PCBs and PBDEs in Toronto. The three Cl-OPEs were TCPP, TCEP and TDCPP. The three non-Cl-OPEs were EHDPP, TBEP and TPhP. Our goal was to estimate their emissions to Toronto and to evaluate their environmental pathways. Aggregate emissions to the air (0-50 m elevation) were estimated by back-calculating from measured outdoor air concentrations. These results were then evaluated against measured water concentrations in Toronto tributaries. Based on estimated emissions to air, modelled water concentrations were within an order of magnitude of the measured concentrations, ranging from 18% below for TCPP to 4600% above for TPhP. With the exception of TCPP, the model over-estimated water concentrations. Since the water concentrations were taken independently of the air concentrations, these results gives some credence to the model estimates and showed that the emissions estimates were accurate to approximately an order of magnitude. The emissions of OPEs ranged from 39 to 400 g/h and were significantly higher than emissions of PCBs and PBDEs calculated using the same model. We found that 70 and 60% of atmospheric loadings Cl-OPEs and non-Cl-OPEs were lost from Toronto by air advection. Eleven % of Cl-OPE loadings were advected from Toronto by surface water in comparison to 5.0% for the non-Cl-OPEs. Transfer from air to water was greater for these highly soluble compounds relative to PCBs and PBDEs. The difference between Cl-OPEs vs non-Cl-OPEs is consistent with their relative differences in water solubility. These model estimates are consistent with the hypothesis of long range transport of Cl-OPEs to the Arctic by rivers. Degradation of OPEs ranged from 11 to 45 % of atmospheric loadings: 18% of Cl-OPEs and 32 % of non-Cl-OPEs were lost to degradative processes. Again, this difference is consistent with estimates of the persistence of Cl-OPEs in comparison to non-Cl-OPEs.

TH099

Ecotoxicological risk assessment of traffic-related non-exhaust emissions

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The demand for transport of both passengers and goods has been growing steadily and is projected to continue within Europe. This increased traffic load does undoubtedly have a major negative effect on the environment and contributes to emissions of greenhouse gasses and noise, as well as local air-, soil- and water pollution. While engine exhaust emissions have been strongly reduced by EU emission standards in the past decades, currently non-exhaust emissions from road vehicles remain unaffected by such measures. Non-exhaust particles typically arise from abrasive sources which include brake wear, tire wear and abrasion of the road surface. Runoff from roads comprises a wide range of metals, hydrocarbons and organic compounds and several can leach to receiving waters and cause toxicity to aquatic organisms. Within the scope of a network of expertise, funded by the Federal Ministry of Transport and Digital Infrastructure, we will investigate the toxic fraction of different non-exhaust contaminants in samples from road runoff, road runoff detention systems and road dust. As a first step, ecotoxic effects caused by compounds eluting from tires will be assessed. Elutes of different types of new and aged tires will be assessed with a battery of methods including organismic in vivo tests as well as mechanistic in vitro assays such as the Yeast Estrogen Screen or the AMES-Fluctuation assay. These data can be used for a relative comparison of the environmental impact possibly caused by tire abrasion. Furthermore, the findings of this study will support the evaluation of the current state of runoff treatment systems, as well as offer incentives for the development of new abatement measures and product design.

TH100

Synthesis of reference substances and method development for the analysis of chlorinated and brominated methanesulfonic acids in environmental and drinking water samples

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Chlorinated and brominated methanesulfonic acids are recently discovered water contaminants that appear to be predominantly present in drinking water samples and are probably derived from water disinfection. A first hydrophilic interaction liquid chromatography-high resolution mass spectrometry (HILIC-HRMS) based semi-quantitative screening estimated a maximal detected concentration of the most prevalent congeners in excess of 100 ng/L. However, the concentrations were calculated against the commercially available trifluoromethanesulfonic acid as standard substance, and therefore matrix effects and differences in the ESI response factors were not taken into account. So far, reliable quantitative analysis of these substances, and thus a comprehensive monitoring, is hindered by the lack of commercially available reference standards. Since chlorinated and brominated methanesulfonic acids are not intentionally produced, neither registration data nor pure substances for laboratory experiments are available, and therefore little is known about their short and long term toxicity. The lack of reliable quantitative information and (eco)toxicology data renders it impossible to thoroughly assess the environmental and drinking water relevance of chlorinated and brominated methanesulfonic acids, and thus it remains unclear if they pose a threat to the aquatic environment or human health. To acquire quantitative data and facilitate future toxicity experiments, we synthesised and purified standards for several chlorinated and brominated methanesulfonic acid congeners in our laboratory. With these standards it was possible to develop the first quantitative LC-MS/MS method for these emerging contaminants. **Acknowledgement** We thank the European Union Joint Programming Initiative "Water Challenges for a Changing World" (Water JPI) and the BMBF for founding the PROMOTE project (FKZ: 02WU1347B).

TH101

ACCURATE MASS SCREENING OF PESTICIDE RESIDUES IN FRESH WATER AND SEDIMENTS USING LC-HYBRID LTQ/ORBITRAP-MS

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Pesticides are extensively used worldwide in agricultural production and they are discharged mainly through run-off from agricultural fields. The irrational use of pesticides has raised concern over the years due to possible pollution posed from their residues. These residues may expose to a high risk some non-target organisms and lower the quality of aquatic ecosystems. Hence, the analysis of such residues even at trace levels in a variety of environmental matrices is necessary to assess the contamination burden in aqueous bodies, preferably in just one run. The present work describes the optimization of the state-of-the-art technique of liquid chromatography coupled to high-resolution Orbitrap mass spectrometry applied to the analysis of pesticides in environmental matrices. HRMS technology is employed as a reliable tool allowing the analysis of a wide range of pesticides in natural waters and sediments. After defining the final optimal conditions, the method was validated for the determination of a broad spectrum of pesticides in natural waters and sediments. The method proved to be accurate and precise with excellent linearity and detection limits for the tested concentration.

TH102

INVESTIGATION OF PSYCHIATRIC DRUGS AND THEIR METABOLITES IN WASTEWATERS AND SURFACE WATERS USING LIQUID CHROMATOGRAPHY COUPLED TO HIGH-RESOLUTION-ORBITRAP MASS SPECTROMETRY

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Detection of pharmaceuticals in the environment has raised concerns in recent years. Nowadays, the higher prevalence of psychiatric disorders led to a worldwide increased number of prescriptions for psychiatric pharmaceuticals. After intake, these highly active compounds undergo metabolic transformations, with subsequent excretion of their active metabolites to raw sewage, wastewater treatment plants (WWTPs) and consequently to surface waters [1]. Since WWTP have been pointed out as the main contamination pathway of pharmaceuticals (and consequently for antidepressants) into the environment, it seems that often only partial removal is achieved in biological treatment processes [2]. In the present study, the distribution of nine psychiatric drugs (fluoxetine, sertraline, venlafaxine, olanzapine, quetiapine, mirtazapine, clozapine, bupropion and paroxetine) and four of their metabolites (norfluoxetine, N-Desmethyl Sertraline, O-Desmethylvenlafaxine and N-Demethyl Olanzapine) in surface waters and wastewaters in Greece was determined. More specifically, an off-line solid phase extraction followed by UHPLC-LTQ-Orbitrap-MS methodology, in positive ionization mode, was applied. Isotope dilution was implemented for every analyte using labeled standards (fluoxetine D5 and olanzapine D3). A number of studies have demonstrated that isotope dilution is a preferred approach in order to alleviate matrix interferences as well as SPE losses and instrumental variability [2-3]. In order to optimize the extraction method, three different cartridges were tested, SDB-1, Oasis MCX and Oasis HLB, and the results showed that most of the compounds exhibited higher recoveries using Oasis HLB.

TH103

A COMPREHENSIVE ANALYTICAL METHOD FOR DETERMINATION OF DECHLORANES AND NOVEL BROMINATED FLAME RETARDANTS IN BIOTA AND ENVIRONMENTAL MATRICES

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The increasing application of halogenated flame retardants led to their widespread distribution in the environment. Recently, concerns emerged regarding their potential persistence, (bio)accumulation and/or toxicity. Particularly Dechloranes like Dechlorane Plus as well as other brominated PBDE replacement, generically called "emerging" or "alternative" flame retardants, are in the focus of interest. In order to assess their background levels in biota as well as environmental trends, an analytical method for the determination of 17 halogenated flame retardants (Dechloranes, brominated aromates, brominated ethers, cyclic BFR) in a variety of environmental matrices (spruce needles, fish, birds eggs, particulate matter) was developed. Samples were extracted using accelerated solvent extraction, followed by a multi column clean-up. Analysis was performed by API-GC-MS/MS as a modern, gentle and sensitive technique for simultaneous detection of compounds throughout a wide range of masses and fragmentation characteristics. Quantification was performed using 8 mass-labeled internal standards. We will present results of validation characteristics as measurement precision, repeatability, intermediate precision, recovery rates of native standard addition, linearity and recovery rates of the used internal standards.

TH104

Identification of emerging contaminants in the environment by LC/Q-TOF MS combined with commercial and open-source MS/MS libraries

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Environmental regulations focus on monitoring a limited number of well-known compounds, representing only a small fraction of the anthropogenic chemicals which can be found in the environment. In addition there are transformation and degradation products formed e.g. during wastewater treatment which are typically not monitored and often not even known. To obtain comprehensive data on the chemical water quality, targeted analytical methods are complemented by untargeted acquisition methods using high resolution LC/MS. Efficient data mining algorithms, statistical software programs and comprehensive accurate mass compound databases and libraries are essential to identify relevant contaminants. Effluents of wastewater treatment plants, receiving water from agricultural and urban areas, were collected as 14-day composite samples. Separation was done using an UHPLC system coupled to a highly sensitive Q-TOF LC/MS instrument. Acquisition was performed with positive and negative ionization with All Ions MS/MS fragmentation. Data was evaluated using targeted data mining tools and statistical software for the non-target screening. Identification of significant features was performed using a commercial accurate mass library combined with information from open source libraries and prediction tools which were made available for the data analysis software by a custom-made library conversion tool. Using the UHPLC Q-TOF LC/MS method for targeted screening for a range of priority pollutants resulted in limits of quantitation in the low ng/L range. A number of pesticides, pharmaceuticals and personal care products were detected in the WWTP effluents and identified based on their accurate mass, isotope pattern and fragment co-elution. In addition multivariate statistics was used to compare the different WWTPs over the sampling period. After data reduction and filtering, significant features in the sample groups were recognized by principal component analysis, hierarchical clustering and similarity searches. Tentative identification was done by MS/MS spectral comparison using both, commercial and open-source libraries as well as in-silico fragmentation tools.

TH105

Novel method optimization for estrogenic activity assessment of steroid hormones in water using DGT and the ERE-CALUX bioassay

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Abstract Adverse effects of steroid estrogens and endocrine disrupting chemicals (EDCs) can occur at relatively low concentrations in the aquatic environment. Monitoring these compounds as encouraged by the European Water Framework Directives (WFD, 2013/39/EU) demands efficient, simple and sensitive techniques to detect and quantify these chemicals. Here, a novel sampling technique of diffusive gradients in thin films (DGT) combined with the chemically activated luciferase gene expression (ERE-CALUX) bioassay was developed for in situ pre-concentration and estrogenic effect measurement of steroids in the aquatic

environment. In the study, the performance of this novel method was assessed, with 17β-estradiol (E2) as a model estrogen in a single compound solution, VM7Luc4E2 cells (formerly BG1Luc4E2) as ERE-CALUX bioassay cells and XAD 18 resin gel as a binding phase. The laboratory tests showed that DGT components and the experimental matrix do not influence the estrogenic activity of ERE-CALUX cells. XAD 18 showed sufficiently high capacity for the binding of E2. The measured effective diffusion coefficient of E2 in agarose diffusive gel was 4.7 $\times 10^{-6}$ cm^2/s at 25 °C, which is slightly lower than the theoretical one (5.2 $\times 10^{-6}$ cm^2/s). The detection limit of this novel method (0.014-0.10 ng/L) was significantly lower than the one obtained from active sampling combined with GC/MS and LC/MS analysis (0.2-7.0 ng/L). This method was independent of pH (5-8), ionic strength (0.001-0.1M) and dissolve organic matter concentrations (0-30 mg/L). This study demonstrates that DGT combined with ERE-CALUX is an effective tool for pre-concentrating steroid estrogens and possibly also in the monitoringofestrogenic activity in natural waters. **Keywords:** Estrogenic activity; DGT; CALUX; Diffusion coefficient

TH106

Virtual Norwegian Environmental Specimen Bank

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The number of chemicals used across the world is increasing fast, and regulatory authorities are struggling to prioritize which chemicals to monitor in the environment. Recent developments in high resolution analytical methods for screening of chemicals may potentially identify a high number of chemicals in the same sample run, compared to the traditional and targeted analyses. In addition, "full scan" chromatograms could easily be stored in databases for retrospective identification of problematic substances in the future. Although such thing as a silver bullet for complete chemical monitoring might not exist, the Norwegian Environment Agency consider high resolution analysis techniques in combination with raw data storage databases to be a cost effective and promising supplement to monitoring. However, several questions need to be discussed; Who owns raw data? Who should have access to it? Who should develop the infrastructure? How can instrument sypplyer specific data formats be handled? Would governmental initiatives compete with already ongoing research driven processes? Norwegian authorities invites the SETAC community to discuss experiences from research and/or governmental environments in the establishment of a virtual environmental specimen bank.

TH107

DETERMINATION OF ILLICIT DRUGS IN SEWAGE TREATMENT PLANTS IN ADANA, TURKEY

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Illicit drug use is an increasing problem in Turkey as well as in the World. Illicit drugs and their metabolites enter the sewage waters by excretion after illegal consumption, accident or intentional extermination. Therefore, illicit drugs are now considered as emerging environmental pollutants. Illicit drug concentrations in influents of sewage treatment plants (STP) can be an indirect tool to estimate the community level of consumption. Adana city is divided into two region by Seyhan River and there are two sewage treatment plants for both regions. This study aims to determine the presence of illicit drugs and their metabolites in two STP which the entire city's waste water comes in and compare each other. In this study, which is one of the first studies in Turkey, presence and daily variations of 13 illicit drugs and metabolites in two sewage treatment plants of Adana was investigated. Waste water samples were collected daily intervals for one week to characterize the weekdays and weekends variabilities of drugs. As a part of this study, a sensitive and selective solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method targeting 13 illicit drugs and metabolites (6-MAM, cocaine, benzoylecgonine, codeine, MDA, MDEA, MDMA, amphetamine, methamphetamine, morphine, THC, THC-OH, THCCOOH) has been developed and used for the analysis. Codein, amphetamine, metamphetamine and Benzoylecgonine were detected in both sampling stations. While cocaine was detected in only 3 samples in Eastern STP, it was detected in all samples in Western STP.MDMA was found in all samples. As an important detail that MDA was only found in Eastern STP at the weekend sampling. 7 of 13 compounds were detected in all samples.

TH108

Studies on Some Persistent Organic Pollutants (POPs) at Nubarashen Landfill

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Soil samples were taken from Nubarashen landfill and analyzed for the determination of 20 organochlorine pesticides (OPs) and 14 dioxin-like PCBs, namely: α-HCH, β-HCH, γ-HCH, δ-HCH, Hexachlorbenzene (HCB), Heptachlor, Aldrin, Dildrin, Heptachlorepoxyl-A, Heptachlorepoxyde-B, p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, o,p'-DDE, o,p'-DDD, Endosulfan-1, Endosulfan -2, Endrin, Mirex, PCB-77, PCB-81, PCB -105, PCB -114 , PCB -118, PCB -123, PCB-126, PCB -156, PCB -157, PCB -167, PCB-169, PCB -170, PCB -180, PCB -189. In most soil samples detected total quantity of HCH isomers exceeded their maximum permissible concentration (MPC) in soil (88,5%). As shown by results of analyses there are samples with HCH isomers concentration 16, 18, 25, 40, 165 times exceeding the MPC. The significant excess of summary standard level occurred mainly due to α-HCH. A similar pattern was observed with HCB. In this case, almost in all samples excess of MPC was observed, and this excess reached up to 10 MPC. In the studied soil samples background amounts of pesticides such as Heptachlor and its epoxy derivatives, isomers of Endosulfan were detected. Occasional cases of Aldrin in soil were recorded in four samples, but the content was below MPC. Isomers of DDT and its metabolites were detected in all the samples, but in quantities below the MPC; only in two samples a slight excess of MPC was recorded. Pesticides Dieldrin, Endrin, Mirex were not detected in any of samples. As eanticipated, in soil samples taken at a landfill, dioxin-like polychlorinated biphenyls were registered. Some PCBs were detected in all samples (PCBs 81 in 100% of the samples), and others in most of the samples (PCB-105 in 80.77% of samples; PCB-118 in 84.6% of samples; PCB-123 in 88.46% of samples). Dioxin-like PCBs 126, 157, 169, 170, 180, 189 were not detected in any of samples. Conclusions: 1. Analyses of samples of soil taken from Nubarashen landfill revealed determined the presence of dioxin-like PCBs, particularly tetra-, penta-, and hexachloro derivatives. Noteworthy is the fact that PCB-81 was determined in all samples, and in one of them there was the excess of total PCBs level is due to PCB-81. 2. Possible, and sometimes frequent processes of the dump non-intensive combustion lead to the formation of not only dioxin-like PCBs, but also cyclic and aromatic polychloro derivatives. Only this can explain the presence of high concentrations of α-HCH and HCB.

TH109

Occurrence and spatial distribution of six endocrine disrupting compounds in surface- and groundwaters of the Romagna area (North Italy)

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Endocrine Disrupting Compounds (EDCs) are raising increasing concern owing to the harmful effects they can pose on organisms, besides their high persistence and continuous introduction in the environment. In this study the occurrence of six EDCs (estrone - E1, ß-estradiol - E2, 17α-ethinylestradiol - EE2, bisphenol A - BPA, perfluooctanoic acid - PFOA, perfluorooctane sulfonate - PFOS) was assessed in the Apenninic rivers and groundwaters of the Romagna area (North of Italy) by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). This is the first report of EDC occurrence in the area, and it is useful to gather information on how far contamination by EDCs is spread in Italian river bodies, in line with EU WFD 2000/60/EC purposes. The study revealed a total absence of EDCs in groundwaters, whereas in surface waters all classes of compounds were detected at concentrations above the method limit of quantification (MLOQ). In particular, PFOA and PFOS were detected at the lowest concentrations (range of < MLOQ – 17.73 ng/l for PFOA and < MLOQ – 5.46 ng/l for PFOS) and they showed a spatial distribution closely related to the discharge of wastewater treatment plants (WWTPs), with the highest concentrations registered downstream of the WWTPs. Moreover, they showed a spatial linear- gradient pattern of concentrations gradually increasing moving from the headwaters to the river mouths. Regarding estrogens, EE2 was below the MLOQ in all samples, whereas E1 and E2 reached concentrations up to 39.74 ng/l (E2) and 28.01 ng/l (E1) in surface waters. The highest estrogen values (> 20.54 ng/l, median value) were located in those areas where livestocks and farming are the main activities, indicating a close relation between these activities and estrogens release in the aquatic compartment. BPA (< MLOQ – 34.72 ng/l) showed a spatial distribution not related to any specific anthropic activity, but rather to a mixture of sources of contamination, such as the various industries of food packaging and plastic production present in the area. Overall, the northern part of the Romagna area resulted to be more affected by the occurrence of EDCs in the aquatic environment, if compared with the southern part, which was less impacted by these micropollutants.

TH110

Emerging contaminants in river water samples from Brazil

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Emerging contaminants, including pharmaceuticals and personal care products, have been investigated in the environment over the past decade. These compounds are not yet regulated in legislation regarding water quality and most are potentialy hazardous to human health and the environment. The purpose of this research was to study the occurrence of pharmaceuticals, hormones and personal care products in surface waters of Brazilian rivers. The determination of the emerging contaminants in surface water was based on solid-phase extraction (SPE, Strata X) followed by analysis via liquid chromatography using a diode array detector (LC-DAD). The chromatographic separations were achieved using a Zorbax Eclipse Plus-C18 column. The mobile phases used were LC-grade water containing 0,1 % acetic acid (eluent A) and acetonitrile (eluent B). The analytical method was applied to water samples from the Mogi Guaçu River in the State of São Paulo and Itapecuru River in the State of the Maranhão. The water of the rivers studied was compared both in terms of the physico-chemical properties and the presence of the emerging contaminants. This study will contribute to the provision of data on the presence and concentration of emerging contaminants in surface waters of Brazilian rivers.

TH111
DETERMINATION OF HUMAN PHARMACEUTICALS IN SUCULLU AND ULUBORLU DAM LAKES (ANTALYA BASIN, TURKEY)
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Human pharmaceuticals are emerging environmental micropollutants and concerns about these substances is increasing day by day. They enter surface waters by many ways: human consumption, excretion, and insufficient removal during sewage treatment. In this study, human pharmaceutical pollution of Sucullu and Uluborlu Dam Lakes were investigated. Water samples were collected as composite samples from stations, seasonally, during the periods of April 2014- December 2014. A sensitive and selective liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method was used for the analysis of 95 pharmaceutical compounds from variety of drug groups including pain killers (analgesics, anti-inflammatories and antipyretics), antibiotics and antibacterial, cardiovascular drugs (β-blockers), hipolipidemics and central nervous system drugs, stimulants has been developed and employed to investigate the occurrence of these pharmaceuticals in water samples. Solid phase extraction techniques were used for all samples. In Sucullu and Uluborlu Dam Lakes; 9 and 15 kinds of human pharmaceuticals were detected respectively. Lidocaine was detected in both lakes in all seasons. The highest measured pharmaceutical concentration was caffeine (Uluborlu Dam Lake in summer, 13.73 ng/L; Sucullu Dam Lake in winter, 6.67 ng/L), which is used as an indicator of anthropogenic impact at surface waters. All concentrations were between low ng/L and low μg/L. But there is no information about the possible effects of pharmaceuticals in case of long time exposure in low dosages and when multiple drugs simultaneously exist. Risks to the environment and human health should be under consideration and further studies should be done.

TH112
Do pit waters from coal mines contribute to PCB contamination of surface waters?
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The Ruhr area in North Rhine-Westphalia, the biggest conurbation in Germany, was a center for hard coal mining since the 1830s. Though coal mining is still only actively operated in two pits in North-Rhine Westphalia, underground water levels have to be controlled and pit waters are discharged into surface waters. In the mines, polychlorinated biphenyls (PCBs) and Ugilec compounds, being temporarily applied as PCB surrogates after their phasing out, had been used in hydraulic fluids. Since there are still hydraulic fluids underground, it is discussed if pit waters contribute to contamination of river waters with PCBs and PCB surrogates. In a previous study, PCB levels were measured in suspended particulate matter obtained from pit waters. This was, however, challenging because the amount of suspended particulate matter in pit waters is very low. Furthermore, ferrum(II)hydroxide precipitates while suspended particulate matter is sampled by centrifugation. In the present study, we applied silicone-based passive samplers to assess contamination of surface waters by PCB residues in pit water discharges. For this purpose, we selected five sites in the rivers Emscher, Lippe, Rhine and Ibbenbürener Aa. In autumn 2016, we exposed silicone rubber sheets for five weeks either directly in pit waters or in surface waters close to the discharge points. Analyte-specific sampling rates were determined by *in situ* dissipation of performance reference compounds (PRCs) that had been spiked onto the passive samplers prior to the field exposure. In parallel to passive sampling, sediments were sampled in surface waters upstream and downstream of discharge points. In the laboratory, passive sampler and sediment extracts were analysed for 7 indicator PCBs (PCB 28, 52, 101, 118, 138, 153, 180) and Ugilec compounds by GC-MS/MS. Time-weighted averaged concentrations (C_{TWA}) of PCBs in water were quantified from analyte concentrations in passive sampler extracts and site-specific sampling rates. Preliminary results show that in or close to pit water discharges C_{TWA} of the sum of 7 indicator PCBs are in the low ng L^{-1} range. These levels are comparable to or sligthly higher than concentrations measured with the same technique in large rivers in Germany. Overall, sediment monitoring and, in particular, passive sampling are very versatile and precise methods for assessing contamination of mine waters with hydrophobic organic contaminants.

TH113
OCCURRENCE OF PHARMACEUTICALS AND PERSONAL CARE PRODUCTS IN KONYA CLOSED BASIN LAKES, TURKEY
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Pharmaceuticals and personal care products referred as emerging contaminants has been considered a big concern because of their adverse effects on non-target organisms in the last few decades. These compounds enter surface waters via many ways; the main pathway from humans ingestion followed by subsequent excretion. In Turkey, there is a lack of data concerning the occurrence and levels of pharmaceuticals and personal care products in lakes. This study assessed regional and seasonal variations of pharmaceuticals and personal care products detected from Ivriz, Ayranci and Godet Dam Lakes in Konya Closed Basin, which located at the middle of Turkey, over the periods of April - December 2014. Solid phase extraction and a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) screening method was developed and used for the analysis of 95 compounds from variety of drug groups including pain killers, antibiotics and antibacterial, cardiovascular drugs, hipolipidemics, central nervous system drugs and stimulants. Recoveries were between 75-115 %. Of the 95 compounds examined, 13 of them were quantified in lake water samples. 7, 11 and 10 different pharmaceuticals were detected in Ivriz, Ayranci and Godet Dam Lakes respectively. Lidocaine and DEET were identified in all lakes and seasons. Caffeine, used as an indicator of anthropogenic impact in the aquatic environment, was detected at the highest concentrations (Ivriz Dam Lake in summer; 20.58 ng/L). Environmental concentrations of these pharmaceuticals are low, but when they behave together may be toxic to the non-target aquatic organisms.

TH114
How far have plasticizers and additives penetrated our aquatic environment?
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Phthalates and phenols are a large group of ubiquitous, high-volume industrial chemicals that are added to plastics and many other daily products, such as building materials, toys, paints, adhesives and medical equipment. Due to the weak physical bonds between a polymer and the plasticizers or additives, they can easily be released directly or indirectly (i.e. through metabolisation, manifacturing processes) in the aquatic environment. Continuous exposure to these substances can exert to toxic, mutagenic, and carcinogic effects on organisms by interfering with their endocrine system. Therefore, monitoring phthalate esters and phenolic compounds in the marine environment, whether or not in the metabolised form, will provide insight into the degree of occurence that these compounds have already reached in our aquatic system. The aim of this study was to develop a multi-residue method for the simultaneous determination of 20 phthalate esters and 10 phenolic compounds in the marine environment. The analytes were separated with an ultra high performance liquid chromatograph (UHPLC) using a 1.9 μm Hypersil Gold column (100 mm x 2.1 mm), and quantified in full-scan by a Q-Exactive Benchtop™ high resolution mass spectrometer (HRMS). Chromatographic variables, such as mobile phase, mobile phase modifier, flow and column oven temperature, were optimised to enhance phenol sensitivity. Additionally, a trap column (1.9 μm, 50 mm x 2.1 mm) was used for eliminating phthalate contamination originating from the analytical instrument. Furthermore, the mass spectrometric variables (such as position source, sheath gas, auxillary gas, sweep gas, discharge current, capillary temperature, S-lens RF, and vaporizer temperature) were optimised. Next, a suitable extraction was designed for capturing a broad polarity range (Log K_{ow} ranging between 1 and 12), including the diphthalates, monophthalates and phenols. Future perspectives comprise the validation of the developed UHPLC-HRMS method according to CD 2002/567/EC and the analysis of phthalates and phenols in the Belgian Part of the North Sea. A major advantage of our newly developed method lies in its ability to identify suspected and unknown compounds, besides the list of aformentioned target compounds. Finally, this work will be an important step towards environmental forensic profiling, future European legislation and a contribution to the Marine Water Framework Directive.

TH115
Measuring emerging organic micropollutants in the North Sea using high-resolution Orbitrap mass spectrometry: method validation and occurrence in harbour and open sea
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The presence and long-term exposure of the aquatic environment to pharmaceuticals, pesticides and personal care products has awakened a growing concern amongst environmental policy makers and researchers. As many of these micropollutants are ubiquitously distributed, many researchers have focused on their occurrence in different matrices such as ground water, riverine water and wastewater. The marine environment has received noticeably less attention so far,

given a.o. the challenges with respect to sampling and ultra-trace (≤ ppb-levels) concentrations of complex mixtures of micropollutants. New monitoring approaches are needed and the use of modern high resolution mass spectrometry offers promising potential for multi-residue ultra-trace analysis of unknown and quantification of known micropollutants. The goal of this study was twofold. First, a novel full-scan SPE-UHPLC-Orbitrap-HRMS method was developed and validated. Second, a simultaneous monitoring of 89 target compounds – including pharmaceuticals, pesticides and personal care products – was performed in the Belgian Coastal Zone (BCZ). Triplicate samples were taken during spring 2016 from 2 different sites in the BCZ; i.e. the harbour of Zeebrugge and roughly 6 km offshore of Zeebrugge. The samples were extracted on an automated SPE-device using Oasis® HLB cartridges; separated through UHPLC on a Hypersil Gold column (50 mm x 2.1 mm, 1.9 μm) prior to Q-Exactive™ Orbitrap HRMS analysis. Results of the validation and monitoring will be presented during the conference. Briefly, the method detection limits ranged from 0.01 ng L^{-1} to 10 ng L^{-1} for 68% of the target compounds. At a concentration of 15 ng L^{-1}, the method precision, expressed as relative standard deviation over triplicate injections, was less than 20% for 64 compounds. Next, a total of 14 and 18 compounds could be quantified offshore and in the harbour, respectively, with concentrations ranging from 0.35 ng L^{-1} to 20 ng L^{-1}. A next sampling campaign is planned for the winter 2016-2017, from which the results will be included in the conference contribution. **Acknowledgments** The authors like to acknowledge the Belgian Science Policy (BELSPO) for funding the NewSTHEPS project (BR/143/A2/NEWSTHEPS). The financial support from the Hercules Foundation (Flemish Government; AUGE/11/016) and from the Ghent University Special Research Fund (01B07512) is acknowledged for the UHPLC-Q-Exactive™ and the automated SPE equipment, respectively.

TH116

Occurrence of oxygenated polycyclic aromatic hydrocarbons in mussels at the Belgian coast

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Oxygenated polycyclic aromatic hydrocarbons (oxyPAHs) are polycyclic aromatic hydrocarbons (PAHs) containing carbonylic oxygen atoms. OxyPAHs are in many cases more toxic than the analogous, non-oxygenated PAHs and therefore, their occurrence in soil and on airborne particulate matter has gained increasing attention. For the marine environment, data are scarce, although Layshock et al. (2010) found that the total concentration of 9 oxyPAHs in standard reference material mussel tissue (SRM2977) was 50% higher than the sum of the 16 US EPA priority PAHs concentrations. A LC-MS^2 based analytical method is developed, validated and applied to determine 6 oxyPAHs (naphthalene-1-ol, 9*H*-fluorenone, anthracene-9,10-dione, 7*H*-benz[de]anthracene-7-one, naphtacene-5,12-dione and benzo[a]anthracene-7,12-dione) in mussel tissue. Reproducibility and accuracy were determined based on 6 non-spiked, 6 low-level spiked (1.66 ng.g^{-1}-2.51 ng.g^{-1} of individual oxyPAH) and 6 high-level spiked (7.6 ng.g^{-1}-11.5 ng.g^{-1}) analyses of a homogenized pooled sample. The reproducibility, represented as the coefficient of variation, was < 4.5% for each compound. Accuracy at different concentration levels was between 86-124%, except for naphthalene-1-ol (52%). For the latter, a recovery factor of 2 was applied. Detection limits varied from 0.42 to 3.57 ng.g^{-1}. Mussels sampled at different quaysides and groynes at the Belgian coast were analyzed on both PAH and oxyPAH concentration. PAH concentrations at quayside mussels were the highest at the most industrialized port of Zeebrugge. In groyne mussel samples, higher PAH concentrations were found at the west, nearby the port of Zeebrugge and the mouth of the Western Schelde, compared to the east or middle of the Belgian coastline. Regarding oxyPAHs, anthracene-9,10-dione revealed the highest concentrations also at the industrialized port of Zeebrugge (19.3 ng.g^{-1} fresh weight), being 6 – 7 times higher than the concentration of the analogous, non-oxygenated PAH anthracene at the same location (2.86 ng.g^{-1} fresh weight). Naphthalene-1-ol, benzanthrone and benzo[a]anthracene-7,12-dione revealed the highest concentrations in the groyne mussels sampled at the east of the Belgian coastline. In conclusion, oxyPAHs show to occur in mussels of the Belgian coast, with concentrations that might be at least as high as that of the commonly measured PAHs which indicates a potential risk to the marine environment related to these emerging contaminants. \n

TH117

Organophosphate flame retardants in L'Albufera Natural Park (Valencia, Spain).

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Organophosphate flame retardants (PFRs) are a diverse group of chemicals added to manufacture materials to inhibit or delay the spread of fire. They are also used as plasticizers, stabilizers, antifoam, humectants, etc. The fact that they are not usually chemically bonded to the material, together with the large volumes consumed and its wide range of applications, raise high concern about the concentrations than they can reach in the different compartments of the ecosystems. The analysis of PFRs in environmental matrices is important for understanding the transport, accumulation and fate of these pollutants in the environment. The determination of PFRs was made by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Therefore, our research focuses in the determination of PFRs in different fish species collected in L'Albufera and the correlation of their concentrations with those found in surfacewaters and sediment. The eleven PFRs selected were tri-n-propylphosphate (TPP), cresyldiphenylphosphate (CDP), tris(2-ethylhexyl)phosphate (TEHP), tris(2,3-dibromopropyl)phosphate (TDBPP), tricresylphosphate (TMPP), tris(1,3-dichloro-2-propyl)phosphate (TDClPP), tris(2-chloroethyl)phosphate (TCEP), tris(2-chloroisopropyl)phosphate (TClPP), triphenylphosphate (TPhP), tris(2-butoxyethyl)phosphate (TBEP) and tributylphosphate (TBnP). To extract PFRs from sediment, and biota, shaking and ultrasonication with methanol followed by solid-phase extraction (SPE) procedure was used. This SPE with STRATA-X cartridges was also applied in water samples. Separation was achieved by means of a conventional analytical column (50×2.1 mm, 1.7 µm) using (A) water - (B) methanol, both with 0.1% formic acid in a gradient. MS/MS was performed in selected reaction monitoring mode with ESI in positive mode. 22 water and sediment samples, 18 sediment samples and 5 different species of fish were analysed. In water, CDP, TClPP and TCEP were the compounds found at higher concentration. In soil and sediment samples high concentrations were found for TClPP, TDClPP and TDBPP. Finally, in biota samples, TClPP was found at the highest concentration. Relationships between high PFRs concentrations and sampling points located downstream wastewater treatment plants were found. This study demonstrates the presence of PFRs in the l'Albufera National Park.

TH118

Chlorinated benzenes in organs of fishes from Lake Dongting

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Chlorobenzenes (CBs) are of worldwide concern due to their toxicity, reports on CBs in aquatic organisms-especially the aquatic organisms in typical epidemic areas of schistosomiasis prevalence in China-are lacking. 68 fish samples were collected from Dongting Lake. The contents of 12 CB congeners in fish samples were measured using GC/MS technique. The results indicated that the concentrations of CBs in muscle were ranged from 2731.5 (yellow catfish) ng/g lw to 7811.2 (catfish) ng/g lw. 1, 3-dichloroben, 1,4-dichloroben, 1,2-dichloroben and HCB were the dominant CB congeners in muscle samples of 3 species fishes. Concentrations of CBs in brain, gonad and kidney were ranged from 2423.2 (crucian carp) to 3329.6 (catfish), 2557.9 (catfish) to 4640.1(yellow catfish) and 1628.1 (yellow catfish) to 4667.8 (crucian carp) ng/g lw, respectively. CBs from the production and use of Sodium pentachlorophenate has been identified as one of the most important sources, which should explain that the high concentration of CBs could be found in organs of fishes from Dongting Lake. CBs cumulative risks for the three species of fish are all slightly exceeding 1 case per million, some consumption advisories should be in place to protect the local residents from further exposure to pollution that may lead to the development of cancer.

TH119

Polychlorinated naphthalenes (PCNs) in Great Lakes fish

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Polychlorinated naphthalenes (PCNs) were one of the first groups of synthesized chemicals, and their high-volume production started around 1910. PCNs were used extensively during the 1930s to 50s, and then phased out around the 1960s (replaced by the supposedly less toxic PCBs) before reliable analytical methods were developed. As such, limited environmental data exist for PCNs, which has resulted in consideration of PCNs as a contaminant of emerging concern. Since resources are limited, it is important to understand the current status of PCNs, and if continued monitoring is necessary. We present a comprehensive view on PCNs in fish from the North American Great Lakes, which hold one fifth of the world's surface freshwater. PCN-42, -52/60, 53, -64/68, -66/67, -69, and -71/72 were the only congeners that were above the detection limits in >70% of total 470 samples. The average congener-specific concentrations ranged from 0.40-92 pg/g ww contributing 0.2-34% to ∑PCN. PCN-52/60 contributed most (34%) followed by PCN-42 (21%) and -66/67 (15%); however, PCN-66/67 were the predominant congeners contributing to 2,3,7,8-TCDD Toxic Equivalent (TEQ) concentration. A limited analysis of short-term temporal change between 2006-07 and 2012 in PCN-66/67 indicated a decline mostly in the range of 24-77%. The PCN-TEQ contribution to total TEQ (i.e., PCNs+Dioxins+Furans+dlPCB) ranged (25th-75th percentile) from 4.7-12%. Based on the findings, a continued regular widespread monitoring of PCNs in Great Lakes fish is not necessary except for certain locations such as the Detroit River. As a cautionary approach, calculated TEQs based on dioxins, furans and dioxin-like PCBs can be increased by 15% to account for the presence of PCNs.

TH120

Exposure Assessment of Residual Organochlorine Pesticides (OCPs) in Paddy

and Upland Soils in Korea
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Residual organochlorine pesticides (OCPs) are chemical substances that are resistant to environmental degradation chemical, biological and photolytic process, and are bioaccumulated with potential significant impacts on human health and the environment. The majority of OCPs were designated as persistent organic pollutants (POPs) by the international community at the Stockholm Convention on Persistent Organic Pollutant. This study was conducted to investigate the OCPs residue in paddy and upland soils. Extraction and clean-up method for the quantitative analysis of OCPs was developed and validated by gas chromatography (GC). Recovery and limit of detection (LOD) of OCPs in soil were 74.4-115.6 and 0.02-0.08 mg/kg, respectively. The precision was reliable since RSD percentage (0.5-3.5) was below 20, which was the normal percent value. The residue of OCPs in paddy and upland soils was analyzed by the developed method, and α-endosulfan, b-endosulfan, and endosulfan sulfate were detected at 2.0-12.0, 1.2-53.1, and 2.2-329.8 mg/kg, respectively. The detection frequency of three compounds were 7 (1.9%), 34 (9.3%) and 108 (29.4%) among 367 samples, respectively. But OCPs in green perilla and green pepper, which are cultivated crops in OCPs detection soil, were not detected. These results showed that the residue in paddy and upland soils were lower level than bioaccumulation occurring.

TH121
Concentrations of neonicotinoid insecticides in honey, pollen, honey bees and honey products in Japan
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Neonicotinoid insecticides are widely used in Japan. Seven neonicotinoid insecticides such as Imidacloprid, Acetamiprid, Thiocloprid, Clothianidin, Dinotefuran, Thiamethoxam and Nitenpyram are popular. Their usage began from the beginning of 1990 and is increasing till 2008 in Japan. Recent annual total usage of seven insecticides is not increasing, approximately 400 tons per year in Japan. However, very little is known about their occurrence, their behaviors and their ecological risk in Japanese environment. Especially, there is little known about the exposure and ecological risk of neonicotinoids to wild bees in Japan though these neonicotinoid pesticides are considered to be one of the reasons for losses of bees in EU, Canada and the US. It should be noticeable that the residual levels of neonicotinoid pesticides in foods are much higher than those in EU and the US and that some news reported that losses of bees and honeycombs occurred recently in Japan. The aim of this research is to reveal ecological risk assessment of honeybees including loss risk of honeycombs in Japan. The risk assessment is conducted by using field monitoring data in Japan. The field monitoring is being performed in two approaches. One approach is to measure neonicotinoid concentrations in honey, honeybees, larvae, pupae, queen bee, pollen collected from in various places in Japan. The exposure levels of honeybee colonies will be estimated by their concentration directly. The ecological risk for pollinators including honey bees can be discussed. The other approach is to measure the concentrations of neonicotinoids in commercial honey products. This approach is much easier to collect many samples than wild honeycombs. The exposure levels of pollinators can be estimated from their honey products. These two data sets can reveal geographical distribution of the risk, differences of neonicotinoid concentration among flower species, source apportionment of neonicotinoids and historical trends of their concentrations of honey products as well as historical trends of their risk to honeybee colonies.

TH122
Development of an HPLC-MS/MS Method to Measure Chlordecone's Metabolites in Livestock Liver
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Chlordecone (or kepone) is an organochlorine pesticide intensively used against banana weevil from 1960 to 1993 in the West Indies.Chlordecone exposure may lead to prostate cancer and may affect development of the foetus and after birth. Nowadays, this pesticide is no longer used but still remains in soils. Chlordecone is detected in food products (vegetables or meat) and the finding concentration is sometime higher than the Maximum Residue Limits (MRL) in application. This molecule can be metabolized in human, pork and gerbil livers. There is no indication of a metabolism in usual consumed species, like beef, chicken or goat. It's crucial to determine which species can metabolize chlordecone in order to collect toxicokinetic informations about this molecule. Chlordecol, one of the major metabolite of Chlordecone, is formed by the reduction of chlordecone thanks to the aldo-keto reductase. Then, chlordecone and chlordecol can be conjugated following the reaction with the glucuronyl transferase. The aim of this work was to develop a sensitive method to determine chlordecone's metabolites in animal livers.The analysis will first be performed in the liver, which is the main place where the metabolism occurs. Actually, there is already a sensitive method by HPLC-MS/MS to determine the concentration of chlordecone in the liver, muscles and fat. This method was developed by the National Reference Laboratory of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES). Nevertheless, regarding chlordecol, the method used by ANSES was applied and poor recoveries were found. A more specific method has been developed to determine the concentration of chlordecol in livestock liver: it involves a better extraction solvent for chlordecol followed by a clean-up acid method. At the end of the development, SPE, d-SPE and QuEChERS were tested in order to get the best recovery for chlordecol. As for the conjugated metabolites, an enzyme called "helix pomatia" was used to determine the fraction of free and conjugated chlordecone. With the help of this method and after the validation step, it will be possible to determine the concentration of chlordecone's metabolites in bovine, caprine and chicken livers.

TH123
Development of cherry tomato analytical reference material for acetamiprid, carbendazim, diethofencarb, pyridaben, pyriproxyfen
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A cherry tomato analytical reference material for pesticide multi-residue analysis(acetamiprid, carbendazim, diethofencarb, pyridaben, pyriproxyfen) was developed by the Korea Institute of Toxicology. The cherry tomato were cultivated in a greenhouse in Korea and were treated before harvest using commercial pesticide formulations applied by spraying with pressure sprayer. All the pesticides were dissolved in water. A representative sample was taken and analyzed by LC-/MS/MS to check if all pesticides were included in cherry tomato or whether any additional spraying was necessary. The cherry tomato(20 kg) was harvested, frozen using a deep freezer, chopped, freeze drying, homogenized and sub-sampled into glass bottles. Ten of these bottles containing five-pesticides were chosen randomly, and analyzed by LS-MS/MS to check for homogeneity. QuEChERS method was used to analyze five pesticides. According to the ISO 35 guide, the analysis of variance using ANOVA test was performed to calculate the within-bottle standard variation(s_{wb}) and the between-bottle standard variation(s_{bb}). The values of s_{wb} and s_{bb} were less than 8.5% of assigned value based on calculation equation specified by the ISO 35 guide. Therefore, the homogeneity of cherry tomato analytical reference material was confirmed that it was in good agreement. The uncertainty(u^*_{bb}) due to inhomogeneity was also calculated as less than 2.5% for all pesticides. The storage stabilities of five-pesticides in cherry tomato analytical reference material at deep freezer(-80°C), freezing(-20°C), cold (4~8°C) and room temperature(20~30°C) conditions were assessed for storage stability. For all target pesticides, the slop(b1) values were smaller than the corresponding values of [$t_{0.95,\, n-2}$×s(b1)], indicating that there were no statistically significant decreases in the concentration of the target pesticides when the cherry tomato analytical reference material was stored at room temperature(20~30°C) for 8 days, cold(4~8°C) for 90 days and deep freezer(-80°C)/freezing(-20°C) for 130 days. Therefore, this cherry tomato analytical reference material containing five-pesticides (acetamiprid, carbendazim, diethofencarb, pyridaben, pyriproxyfen) should be a useful tool for the proficiency test and validation of analytical methods in pesticide residue of tomato matrix or similar matrices.

TH124
Development of cherry tomato analytical reference material for buprofezin, tebuconazole, tebufenozide, tetraconazole, pyraclostrobin
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A cherry tomato analytical reference material for pesticide multi-residue analysis(buprofezin, tebuconazole, tebufenozide, tetraconazole, pyraclostrobin) was developed by the Korea Institute of Toxicology. The cherry tomato were cultivated in a greenhouse in Korea and were treated before harvest using commercial pesticide formulations applied by spraying with pressure sprayer. All the pesticides were dissolved in water. A representative sample was taken and analyzed by LC-/MS/MS to check if all pesticides were included in cherry tomato or whether any additional spraying was necessary. The cherry tomato(20 kg) was harvested, frozen using a deep freezer, chopped, freeze drying, homogenized and sub-sampled into glass bottles. Ten of these bottles containing five-pesticides were chosen randomly, and analyzed by LS-MS/MS to check for homogeneity. According to the ISO 35 guide, the analysis of variance using ANOVA test was performed to calculate the within-bottle standard variation(s_{wb}) and the between-bottle standard variation(s_{bb}). The values of s_{wb} and s_{bb} were less than 11.3% of assigned value based on calculation equation specified by the ISO 35 guide. Therefore, the homogeneity of cherry tomato analytical reference material was confirmed that it was in good agreement. The uncertainty(u^*_{bb}) due to inhomogeneity was also calculated as less than 3.4% for all pesticides. The storage stabilities of five-pesticides in cherry tomato analytical reference material at deep freezer(-80°C), freezing(-20°C), cold (4~8°C) and room temperature(20~30°C) conditions were assessed for storage stability by comparing slop(b1) value to [$t_{0.95,\, n-2}$×s(b1)] value. There were no statistically significant decreases in the concentration of the target pesticides when the cherry tomato analytical reference material was stored at room temperature(20~30°C) for 8 days, cold(4~8°C) for 90 days and deep freezer(-80°C)/freezing(-20°C) for 130 days. Therefore, this cherry tomato analytical reference material containing five-pesticides (buprofezin, tebuconazole, tebufenozide, tetraconazole, pyraclostrobin) should be a useful tool

for the proficiency test and validation of analytical methods in pesticide residue of tomato matrix or similar matrices.

TH125
Identification of fungicides and fungal toxin(Aflatoxin) in grain tea using LC-HRMS
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The purpose of this study is to investigate pesticides and other toxicants contained in grain tea by target/suspect/nontarget screening method using LC-HRMS. The target tea include green tea, chamomile, cornsilk tea, barley tea, cassia seed tea and solomon's seal tea, which are popular in South Korean market. The sample used for chemical analysis were obtained by brewing teas in hot water for 10 min. The pre-treatment for the brewed water sample was based on followed by multi-layer solid Phase Extraction (SPE). The instrument used for the chemical analysis is composed of UHPLC linked to Orbitrap (Q Exactive Plus). As procedures of analysis, first, suspect screening was conducted for the identification of suspicious toxicants by inputting the suspect list for data-dependent MS/MS acquisitions. Second, nontarget screening was performed to collect the evidence for the presence of unknown/unpredicted toxicants in grain tea. Finally, target analysis followed for the confirmation with the standard materials for tentatively identified toxicants via suspect/nontarget screening. As a result from suspect screening, four fungicides were identified by isotope pattern and fragment analysis with help of MS/MS DB (e.g., MassBank, MZCloud) as follow: Pyrimethanil (barley tea), Pyroquilon (cornsilk tea), Tricyclazole (cassia seed tea and barley tea) and Tridemorph (cassia seed tea). Through nontarget screening, Aflatoxin-G2, a metabolite of Aflatoxins was tentatively identified. Aflatoxins are fungi-originated toxins which may cause cancers. This result indicates that fungicides were applied in a grain tea to control plant-derived fungi, but it was not successful, resulting in grain tea lovers exposed to mixture of fungicides and fungal toxin, aflatoxin-G2, as well.

TH126
Fiber optic color sensor for determination of chemical parameters in different water bodies
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The need for reliable new techniques for monitoring of water bodies has led to rapid development of fiber optic sensors (FOS), for the better performance, features, and valid results compared to standard laboratory methods. Standard laboratory methods have certain limiting factors relating to the inability to obtain immediate and *in-situ* data regarding to current state of monitored water body, use of expensive chemicals and complicated chemical analysis, as well as the possibility of errors in the processes of sampling, transport, storage and extraction of the water samples. FOS with its advantages allows there use for detection of various chemical parameters in different water bodies. In this research was applied prototype of color FOS in laboratory controlled conditions. Most important advantages of this type of device are: efficiency, repeatability, reliability, simple use, small size, as well as low cost and fiber optic feature. FOS converts RGB (Red, Green, Blue) color model to HSV (Hue, Saturation, Value) and based on the color intensity of the sample determines the concentration for parameters of analyte interest in water sample. The device was developed and calibrated for measurement of anions orthophosphate, nitrite, sulfate, cation $Cr^{6+}_{(aq)}$ and total chlorine in surface water samples and total chlorine and residual chlorine in swimming pool water samples. Obtained results by standard laboratory methods (UV-Vis spectrophotometer) are compared with results obtained by FOS in order to demonstrate effectiveness and applicability of the sensor. FOS has proven to be very appropriate for use in the laboratory under controlled conditions as low-cost solution to replace expensive standard equipment. Future research will be target on improving the performance of sensors and construction of mobile devices that could be used in the field terrain. The field device would ensure *in-situ* and realtime information on the current state of the examined water body. Acknowledgement: The research has been supported by Ministry of Education, Science and Technological Development of Republic of Serbia under project Development of methods, sensors and systems for monitoring quality of water, air and soil, III43008.

TH127
A STRATEGY TO MITIGATE IN A SUSTAINABLE WAY AMMONIA LOSSES BY VOLATILIZATION IN THE ENVIRONMENT THROUGH COATING UREA IN A SPOUTED BED
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Nitrogen occupies a prominent position among the essential elements for the development of plant species and for increasing agricultural productivity, usually provided through the application of urea. However, the rapid hydrolysis of urea in soils catalysed by urease can result in significant nitrogen loss to the environment, which can reach 78 % in some conditions. The losses are due to leaching, decomposition and ammonium volatilization, causing serious pollution, and increasing costs. Eutrophication is the response to the addition of excess nutrients, which induces explosive growth of plants and algae, the decaying of which consumes oxygen from the water. It is estimated that 50-70 % of all nutrients reaching surface water originate on agricultural land as fertilizer. Another problem is related to the volatilization to the atmosphere, which can contribute to acidic deposition, formation of tropospheric ozone, or act as greenhouse gases. Many strategies are being developed in order to minimize losses of nitrogen. The coated urea is among these strategies, also known as slow and controlled release fertilizers. The controlled release fertilizers are obtained by coating with low permeability material. The production of coated urea in a spouted bed using a biopolymer is an environmentally benign technology, as it does not need a toxic organic solvent, has high stability, flexibility to chemical modification, low cost and biodegradability specific. Therefore, the aim of this work was to formulate a biopolymer suspension that can be used in the coating of urea and to evaluate the influence of the operating conditions on the decrease of nitrogen volatilisation for the coated urea in a spouted bed. The experiment proceeded in eleven coating tests adjusting the desired conditions of air flow rate and temperature. The results showed that the coating film allowed a decrease in nitrogen volatilization losses for all of the experimental conditions, where the volatility reduction percentage was in the range of 11 and 50 %. The micrographs showed that the coating involved the urea particles as expected, demonstrating that the coating film was effective in controlling nitrogen release contained within the particle.

Hazard and exposure assessment of chemical mixtures: steps towards increasing the realism of human and ecological risk assessments (P)

TH128
Exploring the synergistic potential of azole fungicides in Gammarus pulex
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Azole fungicides are known to act synergistically with other chemicals and can thereby enhance the effect of toxic substances in different organisms. The anti-fungal activity of azoles results from their ability to inhibit the cytochrome P450 (CYP) catalysed ergosterol biosynthesis in fungi. In non-target organisms, CYPs are one of the most important enzyme classes active in drug metabolism and thereby in the detoxification of xenobiotics. Thus, azole fungicides can affect the biotransformation (BT) and bioaccumulation of other chemicals by inhibiting CYP catalysed BT reactions. Synergy in mixtures containing an azole is well-known but is mostly based on effect measurements at high concentrations. Therefore, we aimed to find evidence that the observed synergism of azoles in mixtures is caused by the inhibition of CYPs. To answer this question, the internal concentrations of a selected substrate (the strobilurin fungicide azoxystrobin) and associated biotransformation products (BTPs) were measured in the presence and absence of an azole via online solid phase extraction coupled to liquid chromatography high resolution tandem mass spectrometry in the test species *Gammarus pulex*. Seven azoles (cyproconazole, epoxiconazole, fluconazole, ketoconazole, prochloraz, propiconazole, tebuconazole) were selected and tested separately. In total, 18 BTPs were identified for azoxystrobin and BT predominantly took place via oxidation and/or conjugation reactions. Most BTPs are characterized by changes at the active (E)-methyl β-methoxyacrylate group. Thereby, BT contributed to the detoxification of azoxystrobin. Out of the tested azoles only prochloraz showed a strong inhibitory effect at the chosen concentrations. The internal concentration of azoxystrobin after 24 h exposure was approximately doubled in gammarids co-exposed to prochloraz compared to the internal concentration of gammarids exposed only to azoxystrobin. Consequently, co-exposure to prochloraz lead to increased mortality. As expected, only oxidative BT reactions likely to be CYP catalysed were influenced by prochloraz. Moreover, different approaches were investigated to determine the half maximal inhibitory concentration of prochloraz as a measure for its inhibition strength. Since aquatic organisms are usually exposed to a mixture of numerous pollutants, it is likely that co-occurrence of azoles with other pollutants can lead to increased toxicity of toxic compounds already at environmental concentrations.

TH129
Review of case studies on the human and environmental risk assessment of chemical mixtures
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Humans and wildlife can be exposed to an infinite number of different combinations of chemicals in mixtures via food, consumer products and the environment. The number of chemicals and composition of chemical mixtures one might be exposed to is often unknown and changing over time. To gain further insight into current practices and limitations of mixture risk assessment (MRA),

peer reviewed literature was searched for case studies. The aim was to find examples of MRAs in order to identify chemical mixtures of potential concern, methodologies used, factors hampering MRA, data gaps, and future needs. 21 case studies of human and environmental MRAs were analysed. Several compound classes and environmental media were covered, i.e. pesticides, phthalates, parabens, polybrominated diphenyl esters (PBDEs), pharmaceuticals, food contact materials, dioxin-like compounds, anti-androgenic chemicals, contaminants in breast milk, surface water, ground water, drinking water, and indoor air. Several of the case studies revealed a concern due to combined exposure especially when considering specific vulnerable population groups. Several parameters that could lead to an over- or underestimation of risks were identified. However, there is clear evidence that chemicals need to be further addressed not only in single substance risk assessment. Several issues hampering MRA were identified, e.g. the composition of the mixture in terms of chemical components and their concentrations need to be known. Exposure data are often lacking and need to be estimated based on production and use/consumption information. Moreover, relevant toxicity data are not always available. Reference values used in single substance risk assessments can be found for several chemical classes, however, they are usually derived based on the lowest endpoint. Refined MRAs for specific cumulative assessment groups are often hampered by a lack of specific toxicity and mode of action information. Most of the mixtures addressed in the identified case studies examined specific chemical groups. Only few of them looked at mixtures comprising chemicals regulated under different legislative frameworks. The examples indicated that there is evidence for combined exposure to chemicals regulated under different legislation as well as evidence that such chemicals can elicit similar effects or have a similar mode of action. MRA across regulatory sectors should therefore be further investigated.

TH130

Challenges and Chances in Regulatory Ecotoxicological Mixture Toxicity Assessment for Plant Protection Products

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Over the years, environmental risk assessment of plant protection products (PPPs) became more complex in order to represent ecosystem realities. In this course, now a point is reached where a step forward is made from the established 'one-chemical-at-a-time' risk assessment to the attempt to address the presence of mixtures of chemicals. These mixtures may consist of active substances from PPPs containing more than one active substance or of active substances resulting from the sequential or simultaneous use of different PPPs. For the evaluation and authorization of PPPs in the EU, mixture toxicity is already considered to a certain extent e.g. in Regulation (EC) No 1107/2009 (PPPs 'shall not have any harmful effects…taking into account known and expected cumulative and synergistic effects' and later on that 'interaction between the active substance, safeners, synergists and co-formulants shall be taken into account') and Regulation (EC) No 284/2013 ('any information on potentially unacceptable effects of the plant protection product on the environment…as well as known and expected cumulative and synergistic effects' shall be included).\n Recently, the European Food Safety Authority (EFSA) has launched several activities with regard to the risk assessment of mixtures which should result in a draft MixTox Guidance Document in 2018. Hence, mixture toxicity approaches were already part of the EFSA Guidance Documents on birds & mammals (EFSA Journal 2009;7(12):1438), bees (EFSA Journal 2013;11(7):3295), and aquatic organisms (EFSA Journal 2013;11(7):3290) and of the EFSA Scientific Opinions on non-target arthropods (EFSA Journal 2015;13(2):3996), soil organisms (EFSA Journal 20YY;draft, 248 pp. doi:10.2903/j.efsa.20YY.NNNN) and non-target plants (EFSA Journal 2014;12(7):3800). Therefore, an overview will be given over current mixture toxicity approaches already implemented in regulatory risk assessment for PPPs as well as planned and discussed ones. The following questions are addressed: Differs mixture toxicity methodology among organism groups and are there uncertainties which hinder regulatory mixture toxicity assessments? Is the need for refinement addressed and what are possible refinement options? Where lay advantages and disadvantages? Is mixture toxicity assessment manageable with regard to time and complexity and expectable output? Which country- or zonal specific methodologies exist and do they provide potential for an EU-wide harmonization?

TH131

Evaluation of hazard-relevant metal impurity profiles in chemicals derived from natural source materials

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Metal content profiles vary in dependence from natural source materials from different mining places due to differences in the local geology. This holds true for metal salts derived from ores but also for natural gypsum and as well as for gypsum from Flue Gas Desulphurization (FGD), i.e. a process to remove sulphur from flue gas coal-fired power plants. In variability in FGD-gypsum is caused by the different coal materials used, from which the metals are carried through. As required in the REACH registration, impurity profiles at least potentially relevant for hazard classification have to be reported in the registration dossier and the full range of the possible concentrations of each individual impurity has to be stated. A number of metals have been characterized as CMR substances, among them bioessential ones, e.g. Cobalt(II) salts (Press release ECHA/PR/11/27, 21 December 2011). The 'concentration limit', meaning a threshold of any classified impurity that may trigger classification (REGULATION (EC) No 1272/2008, CLP, Article 2, 32, Official Journal of the European Union, page L 353/10)., will not be reached for any of the impurities known from both gypsum materials. Nonetheless it may be possible that the sum of the individual upper of ranges of all CMR metals may potentially reach this 'concentration limit'. During an authority evaluation this point as been touched. Therefore the underlying thoughts and principles are discussed. Finally it is suggested to use for such summation 50th percentile values, but only when individual analyzed samples the sum of the actually contained weight % of the relevant CMR metals were present in comparable magnitude.

TH132

Pesticides in paddy soil and rice from the Kingdom of Thailand: Fate and health risk assessment

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Paddy rice is overwhelming in developing countries where agricultural sector is the major source of income. A wide range of pesticides is applied extensively in order to enhance crop yield. The impact of pesticides not only affects the crops and environment but also have a negative potential threat to human health. In particular, organochlorine pesticides (OCPs) can accumulate in soil and transfer into plants. The purposes of this study were: (1) to investigate the level, composition, and possible transformation of OCPs in soil and rice from paddy field, (2) to conduct a preliminary health risk assessment based on the human exposure levels of the target OCPs. Twenty-three rice and soil samples were collected from paddy fields in northern (Khamphaengphet province) and southern (Suratthani province) part of Thailand, in October 2015. Five subsamples (within 20 cm from the surface) were taken randomly from an area of 5000 $m^2$and mixed to form a composite sample. Twenty-five OCPs which were commonly used in paddy fields in Thailand were selected. Analytical method; extraction step of paddy soils and grains of rice (5.0 g each) were extracted using sonication. A purification step; the paddy soil extract were loaded into an alumina cartridge and a florisil cartridge, respectively. For paddy rice, gel permeation column instead of alumina cartridge was used for the first step of clean-up. Finally, the eluant was analyzed by an Agilent 6890N GC-ECD. Results indicate that the residue level of OCPs in paddy soils ranged from 3.58 to 34.73 ng g^{-1}dw (dry weight) (mean: 10.24 ng g^{-1}dw). The residue level of OCPs in paddy rice was found ranging from 6.51 to 109.10 ng g^{-1}dw (mean: 25.12 ng g^{-1}dw). Considering DDT and its metabolites; o,p'-DDE, p,p'-DDE, p,p'-DDD in paddy rice were significantly higher than those in paddy soils ($p< 0.05$). Remarkably, only mirex, is significant between OCPs levels in paddy soils from southern and northern part of Thailand. Furthermore, the most structure of OCPs data was similar in the paddy field analyzing by factor analysis. It can be concluded that excepting mirex, other OCPs were similarly applied in northern and southern paddy fields in Thailand. According to health risk assessment, the chronic HQ values of heptachlor epoxide (isomer α-, β), the drins (aldrin and dieldrin) were 13% and 16% following female and male consumer's exposure were 12% and 14%, respectively, suggesting the risk should be considered.

TH133

Pesticides do rarely come alone, except in risk assessment - Typical treatment regimes

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In the European Union, legislation so far strictly regulates plant protection by means of chemical pesticides on the level of single products. Common agricultural practice and manifold pest pressures lead to simultaneous or short-sequenced applications of numerous active substances as combination products or tank mixtures over the complete growing period of a crop. This results in the exposure of non-target organisms to complex mixtures in a spray series. Therefore, it is mandatory to investigate the protectiveness of the environmental current risk assessment (ERA) scheme under realistic treatment regimes. In an on-going project, we established a dataset for the actual application patterns of pesticides in 12 important crops in terms of acreage and pesticide application rates between 2007 and 2015. In total, about 1200 sprayseries from Germany, Italy, Spain and Austria have been collected and validated for plausibility. Combining all preliminary information on empirical use patterns and their regional and temporal variation, we calculated widely accepted indicators of use intensity and hazard potential of an application event. The indicators integrate the complex patterns into one result and thus allow for ranking sprayseries and classifying typical best and worst-case use scenarios. The differing toxicological sensitivities of aquatic and terrestrial test organisms cause major difficulties in finding compromise scenarios that can be deemed worst or typical for all areas of ERA. Nevertheless, our results emphasize the relevance of pesticide mixtures for the exposure of non-target organisms. In order to derive an (screening) indicator for comparative hazard assessment of PPP spray series, we combined available information on application rates and the toxicity of single active substances. We clearly see the necessity to integrate realistic exposure assessments of typical treatment regimes as well as effect estimates from appropriate mixture toxicity models in the next steps.

TH134

Addressing chemical mixtures in fish consumption advisories

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Fish consumption advisories are issued to prevent exposure to contaminants beyond what is considered safe. Typically the advisories do account for the presence of multiple contaminants in fish mainly through their Tolerable Daily Intakes (TDIs); however, the final advice issued is based on a contaminant that presents the highest risk to human consumers. In this process, potentially additive or synergistic effects of a chemical mixture are neglected, which could translate into an underestimation of health risk. We investigated how fish consumption advisories for the North American Great Lakes could be impacted if we assume an additive effect of chemical mixture in fish. North American Great Lakes contain more than 140 fish species and are valued at >$5B for recreational and commercial fishing. There is a long history of industrial pollution around the Great Lakes resulting in issuance of fish consumption advisories. Using monitoring data collected by the Government of Ontario, Canada, we simulated advisories using the most restrictive contaminant (one-chem) and multi-contaminant additive effect (multi-chem) approaches. The simulated advisories were then compared to examine the impact of the multi-chem approach. The results showed that about half of the advisories presently issued would be more stringent. Many fish popular for consumption (e.g., Salmon, Walleye, Bass and Trout) would have noticeably more stringent advisories. The results highlight that the health of humans consuming fish from the Great Lakes may not be appropriately protected from exposure to the chemical mixtures.

TH135

Toxicity of Quaternary Ammonium Compounds (QACs) to aquatic non-target microorganisms: tests with single compounds and mixtures

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The toxic effects of five quaternary ammonium compounds (QACs) widely used as active ingredients in personal care products were assessed towards the bioluminescent bacterium *Aliivibrio fischeri* (Microtox® test system). Experimental results showed a relevant toxicity for almost all of the single QACs, with IC_{50} values lower than 1 mg L^{-1}. The comparison between experimental and predicted IC_{50} values from the Quantitative Structure-Activity Relationship (QSAR) models indicated an a-specifically reactivity of most of the selected QACs to the test organism. Only hexadecyl trimethyl ammonium chloride (ATMAC-16) behaved as a polar-narcotic, with a low reactivity towards the bacterial cell membrane. The concentration response curves of different binary and multicomponent mixtures of QACs were also evaluated with respect to the predictions from the Concentration Addition (CA) and Independent Action (IA) models. For almost all the binary and multicomponent mixtures (7 out of 11 tested mixtures) a good agreement between experimental and predicted IC_x was observed, as confirmed from the application of the Model Deviation Ratio (MDR). In four cases, some deviations from the expected behaviour were observed (potential antagonistic and synergistic interactions) at concentrations in the order of hundreds μg L^{-1}, which could be of environmental concern especially in the case of synergistic effect. The analysis of aquatic ecotoxicity data and the few available values on measured environmental concentration (MECs) from literature for wastewaters and receiving waterbodies suggests that a potential risk for aquatic life cannot be excluded.

TH136

Synergic effects of surfactants on the toxicity of carbamazepine and triclosan in aquatic vertebrates and invertebrates

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Arguably, the main limiting factor for the bioavailability of a contaminant is its water solubility. Surface-active substances, capable of modifying the solubility of organic compounds, may act as a vehicle for certain pollutants enhancing their bioavailability and increasing toxicity to aquatic organisms. In this study, carbamazepine (log Kow 2.45) and triclosan (log Kow 4.76) were selected as representative of emerging contaminants to study the synergic toxicity effect with the fluorosurfactant perfluorooctane sulfonate (PFOS) in two biological models, Daphnia magna and the medaka fish (*Oryzias latipes*), respectively. The effect of PFOS (6 μg/ml) on the toxicity of carbamazepine (25–100 μg/ml) and triclosan (100 –800 ng/ml) was determined in young daphnids and in early life stages (ELS) of medaka, respectively. For the daphnia bioassays, acute (i.e. immobilization) and sub-acute (i.e. *chlorella* ingestion) endpoints were studied. For the medaka bioassays, a battery of non-invasive biomarkers during embryo and eleutheroembryo development was analyzed. The combined exposure of carbamazepine (≥ 25 µg/ml) with PFOS reduced the chlorella ingestion rates in Daphnia magna although no effects were seen in the immobilization studies. Results from the medaka ELS revealed that a combination of triclosan (≥ 200 ng/ml) with PFOS exerted a synergic effect on different biomarkers of exposure and toxicity during embryo and eleutheroembryo development. This work was made possible by Spanish Government Grants CTM2013-44986-R and CTM2014-52388-R.

TH137

Exploring community based environmental hazard assessments of mixtures based on mode-of-action based approaches

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Conventionally, the complexity of environmental systems is accounted for in risk assessments through assessment factors applied to hazard ratios. Recent years, however, have seen a strong drive in the field of ecotoxicology to move from assessment factors towards approaches that try to elucidate what's behind assessment factors and so to explicitly account for the complexity of environmental systems. Here we consider both the chemical and the ecological complexity that is found when testing communities for the effects of environmentally realistic exposure patterns e.g. in meso- and microcosm experiments. Many data are available for single species exposed to single chemicals, but the pool of data for multiple species exposed to multiple chemicals is limited. To account for chemical complexity, we shortly summarize the state of the art in understanding mode of action (MoA) and methods for assessing mixture toxicity. To address ecological complexity, we review recent developments in models for extrapolation from single species effects to community level effects. In order to evaluate the feasibility of risk assessment approaches that can integrate both chemical and ecological complexity, we present an overview of experimental studies that provide information on mixture toxicity observed at community levels across different chemical MoAs. These studies were categorized according to the applied experimental approach, test substances, test duration, measured endpoints, frequency of measurements, qualitative outcomes and the exposure conditions. This data compilation is used to define potential case studies that will aim to evaluate the compatibility of available data with currently available models for extrapolation to community level effects. Lastly, we present a simple predator-prey model as an example for how modelling can help to extrapolate from single to multiple species. Results of this example show that additivity of a binary mixture observed in single species tests does not guarantee that effects of this mixture on population biomass density will be additive at the community level as well. Based on our analysis, we discuss the feasibility of an integrated risk assessment approach that combines MoA considerations with mixture toxicity to extrapolate to chemical effects at a community level in aquatic systems.

TH138

Micropollutants in European Rivers: A Mode of Action Survey to Support the Development of Effect Based-Tools for Water Monitoring

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environmental research - UFZ / Effect Directed Analysis; R. Altenburger, UFC Centre for Environmental Research / Bioanalytical Ecotoxicology
Environmental quality monitoring of water resources is challenged with providing the basis for safeguarding the environment against adverse biological effects of anthropogenic chemical contamination from diffuse and point sources. While current regulatory efforts focus on monitoring and assessing a few legacy chemicals, due to progress in analytical techniques many more anthropogenic chemicals are and will be detected in our aquatic resources. Assessing this type of exposure information based on available standard approaches from single chemical prospective risk assessment leads inevitably to indication of risk in most surface water bodies (Malaj et al. 2014, Stehle and Schulz 2015). However, exposure to neither individual chemicals nor mixtures does necessarily translate into adverse biological effects. As an alternative to generic assessment approaches effect-based monitoring approaches are suggested (Escher et al. 2014). To become a credible complement to chemical monitoring information we need, however, better understanding of the capabilities and gaps of available effect-based tools (EBT). In this work we therefore undertake to (i) compile organic contaminants detected in freshwater monitoring studies, (ii) provide a synopsis on the mode-of-action knowledge available for the detected compounds to map against available EBTs coverage, and (iii) utilise a hazard identification approach to identify priority compounds for effect-based monitoring. From our work it emerges that chemical occurrence in European freshwaters seems to be highly variable in composition and relative abundancies. Further, while we are substantially limited in our mode-of-action knowledge we can already identify major gaps in coverage of potential effect qualities when relying on established EBTs. Finally, we suggest a list of organic compounds that could serve as a reference list for EBT validation studies (Busch et al. 2016, ET&C *Focus* 35:1887–1899).

TH139
Assessment of acute toxicity of industrial effluents using Daphnia magna
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The industry is increasingly challenged with problems of emissions control of toxic substances into the environment, particularly in the form of liquid effluents. In Sachin (Gujarat, India), the Sachin Industrial Area is Asia's second largest industrial settlement in terms of area. It has various manufacturing units and is one of the areas zoned for industrial development. The town has good location as result of which many industries were established by Gujarat Industrial development corporation (GIDC). These industries are producing huge amount of effluent in treated or untreated form which drained into different creeks nearer to those industries. Due to the discharge of untreated or partially treated effluent into open areas, the ground water and the drinking water are getting polluted. In wastewater quality monitoring, toxicity evaluation is an important parameter which gives comprehensive response of test organisms to effluent. *Daphnia magna* was chosen as the test system because it is the most commonly used zooplankton in toxicological tests and it plays an important role as a food chain organism. The objective of this study was to evaluate the acute toxicity of effluents from different locations of industrial area of Sachin. Total 9 samples (3 samples per season) were assessed for toxicity to *Daphnia magna*. The immobility of daphnidswas determined after 48 h. The clinical symptoms observed were lethargy, at the bottom of the test vessel, floating at the surface of water, lethargy, and immobilization. The highest and the lowest EC_{50} observed were 24.58 and 0.04% v/v, respectively. The clinical symptoms and EC_{50} value indicates that the effluents have very toxic effect on *Daphnia manga*. These industries are responsible for the largest part of the overall toxicity of the effluents. The current research provides information on the potential ecotoxicological risks caused by the effluent to *Daphnia magna*. Based on the obtained results and regarding the improvement of water quality standards, it is necessary to consider more stringent water quality policies for regular monitoring and toxicity assessment.

TH140
Toxicity of complex mixtures of emerging pollutants in groundwater systems
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Groundwater contamination is one of the most important environmental threats resulting from anthropogenic activities. Besides constituting the primary source of drinking water, groundwater aquifers are vital for irrigation in agriculture, industry and for sustaining natural ecosystems functioning and health. Groundwater aquifers are thus limited resources, often overlooked and highly prone to deterioration. Groundwater Directive (2006/118/EC) was created to protect groundwater bodies from contamination by setting quality standards and establishing threshold limits for a set of priority pollutants. However, an extensive group of emerging contaminants whose effects are largely unknown, such as pharmaceuticals, personal care products, engineered nanomaterials and novel pesticides or industrial chemicals, still has no regulatory status which has raised concerns over human and environmental health. Besides, these compounds normally appear as complex mixtures, comprising multiple compounds of different chemical classes and their metabolites. Understanding and predicting the toxicity and behaviour of such mixtures is of paramount importance to adapt groundwater regulation to these new challenges and to develop sustainable management strategies. The present work was performed in the context of the European Research Project WE-NEED (Water JPI- WATERWORKS2014 ERA-NET COFUNDED CALL) focused on developing new management strategies to sustainably exploit groundwater resources. A framework was conceived to identify, characterize and evaluate the toxicity of emerging contaminants in groundwater aquifers. The thorough identification of emerging contaminants took place in two well-characterized case-studies. Based on this step, the toxicity of realistic mixtures could be assessed using a stepwise approach where increasingly complex exposure scenarios are evaluated. These assessments were carried according to standardized protocols for *Daphnia magna* (immobilization and reproduction) and *Danio rerio* (Fish Embryo Toxicity Test) using several pharmaceuticals as emerging pollutants. Empirical information is thereafter integrated and modelled using the reference model of Concentration Addition to detect deviations to additivity that may indicate interaction between contaminants. The consequences for groundwater management are discussed.

TH141
Building spatial composite indicators to identify environmental health risk areas in Walloon region
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Developing an environment and health information system has become a major focus for public health as stressed in the Belgian National Environmental Health Action Plan (NEHAP). In Walloon region, in the framework of the Program of Regional Actions in Environment and Health (PARES, in French), ISSeP works on building an integrated approach of environmental exposure assessment and has developed a spatial tool based on Geographic Information Systems (GIS). This tool aims to identify, on a regional scale, geographic areas where hotspot exposures are a potential risk to human health. Two kind of environmental indicators are used for spatial analysis: risk indicators (RI) and pressure indicators (PI). The first ones are based on environmental measure of pollutants in ambient air and soil, and on nuisances for citizens related to noise and radon. The environmental data are normalized according to reference values quoted by the OMS or Walloon legal limit values, and risk indicators are calculated per pixel. The second indicators provide an estimation of the local pressure related to old or recent pollution sources (roads, industries, landfills, EMF, mine heaps, etc). Pressure indicators are calculated with specific GIS processing which vary in relation to pollution effect to population (i.e. a buffer distance determined according to the literature). Spatial composite indicators are then computed by using a scoring system to weight and sum each set of indicators, with taking presence and characteristics of population in account. By detecting vulnerable populations and analyzing exposure determinants at a fine resolution, the spatial tool developed in this study aims to assess areas where environmental and social health inequalities are accumulating in Walloon region. Finally, it will support decision makers in better understanding, analyzing and managing the health of the population in relation to environmental pollutions and stressors.

TH142
Adaptation of the WHO/IPCS framework on mixtures assessment to regulatory environmental risk assessment practices
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Environmental risk assessments (RA) for regulatory objectives, such as selecting high-priority chemicals, identifying chemicals to be regulated or setting criteria/standards, have in general been conducted on a chemical-by-chemical basis. Typically a single chemical has been identified for such regulatory environmental RA. Our study's goal is how "assessment of combined exposure to multiple chemicals" (in other words, mixtures assessment) could be introduced into the conventional environmental RA practices. The framework proposed by WHO/IPCS in 2011 with tiered approach should be useful considering regulatory mixtures assessment, while it focuses on RA of human health, not of the environment. We have been conducting case studies on environmental RA to see how we should adapt the proposed framework to our regulatory environmental RA practices, from screening-level to comprehensive ones. Updated situation of the case studies and a proposal of adapted tiered framework will be presented at the Annual Meeting.

TH143
Human health Risk Characterisation of inorganic complex materials in the workplace
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Inorganic UVCBs (iUVCBs, Substance of Unknown or Variable composition, Complex reaction products or Biological materials) raise challenges during hazard and risk assessment under REACH and CLP. For the purpose of risk assessment, the hazards of each constituent are assessed and the DNEL/PNEC values for all the constituents for which a hazard has been identified are considered. Potential combined exposure of the iUVCB constituents are assessed in a tiered way: the first

conservative tier is based on the summation of the associated RCRs (Risk Characterisation Ratios). This does not seem to lead to realistic estimates (e.g. due to cumulating pre-cautionary assessment factors) and efficient solutions in terms of risk management. This poster presents a pragmatic way forward to combined human exposure and risk characterization under REACH based on ECHA Guidance for assessing human risk of biocidal products (mixtures). Subsequent tiers consider target organ toxicity and mechanistic data and adds RCRs by target organ. A specific case study is presented for the workplace and demonstrates that combined exposure and risk can pragmatically be assessed in a regulatory context awaiting while science makes progress on combined toxicity assessments. The concept is equally valid for linking human and environmentally oriented assessments.

TH144
A Case Study on Current Risk Characterization Methods for Occupational Exposure to Chemical Mixtures
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The focus of chemical risk assessment under chemical regulations in the European Union (EU) is extending from chemicals to mixtures because mixtures may result to combined effects of toxicity even at no observed effect concentrations (NOECs) in individual components. Despite the urgent need for developing appropriate risk assessment methods in mixtures especially for industrial workers, few studies have been conducted. By taking into account both hazard and exposure data, chemicals risks are in general determined under EU chemical regulation, e.g., Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH). Risk characterization is the last step in the risk assessment to estimate whether or not, risks are properly controlled by calculating the risk characterization ratio (RCR), which is the ratio of the estimated exposure and the derived no-effect levels (e.g. DNELs).If the RCR does not exceed one, it can be regarded that the risk is adequately controlled within given conditions described in the exposure scenario. There are two main technical guidelines for the risk characterization of mixtures: Lead Component Identification (LCID) and Cumulative RCR method. The LCID method is to select the lead component and grouping components based on their similarity. The cumulative RCR method is to use the summation of RCRs of all mixture components having a same exposure route. The objectives of this study were to conduct a comparative case studyon those two risk characterization methods for occupational exposure to chemical mixtures; and to examine possible deviations between results estimated by the two methods, and to derive future challenges in the mixture risk assessment. Occupational exposure and hazard data for mixtures were collected from previously published publications.

TH145
Pesticides do rarely come alone, except in risk assessment - Outline and first results of the project COMBITOX
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Many plant protection products (PPPs) that are authorized in the European Union for agricultural and private use consist of more than one active ingredient. Combination products with several active ingredients are frequently used with other PPP´s in tank mixtures for a series of field applications. Therefore, the assessment of mixture toxicity effects under realistic treatment regimes presents an essential part for the ecological risk assessment of PPPs. Since decades, many studies have addressed the effects of toxicant mixtures on various organisms and endpoints. The model of concentration addition (CA) seems in most cases able to predict the joint effects of mixtures. Nevertheless, there is still a lack of understanding with regard to the predictability of mixture toxicity for chronic effects and effects at higher levels of biological organization in different environmental compartments. For this, the project COMBITOX aims to evaluate existing knowledge on chronic as well as aquatic and terrestrial community mixture toxicity. In addition, a unique and large data set of PPP spray series from different agricultural crops in Germany is analysed to determine typical treatment regimes and their potential impact on non-target organisms. Finally, available models and approaches are critically reviewed for their usefulness of predicting the combined risk of treatment regimes for terrestrial and aquatic ecosystems. Overall, COMBITOX addresses the protectiveness of existing risk assessment schemes based on single products and will discuss solutions within the frame of the existing or future risk assessment of PPPs.

TH146
Eco-friendly Product Design Support Tool (Eco-PDS) for Screening Mixture Toxicity of Chemical Products
J. Kim, H. Jeong, KIST Europe / Environmental Safety Group
The paradigm shift of chemical risk assessment is in progress from ‘chemical safety focusing on single chemicals (i.e., ingredients)’ to ‘product safety extending to mixtures and articles (i.e., final products)’. This is due to the fact that mixture toxicity effects can be provoked among chemicals existing even at levels below no observed effect concentrations. However, requiring to conduct toxicity tests for all conceivable mixture products is unfeasible due to the extremely large number of combinatorial complexity. A need for developing computational toxicology methods as cost savings, fast and reliable approaches to achieve the new paradigm and enhance its workability. In this study, Eco-friendly Product Design Support Tool (Eco-PDS) for estimating the mixture toxicity in a web-based form was developed. Eco-PDS provides the chemical industry with a possible solution to calculate the mixture toxicity of chemical products before conducting experimental toxicity tests. In the tool, two conventional models as the first tier models, and two advanced models as higher tier models, were combined to predict the mixture toxicity in four different models. Concentration Addition (CA) and Independent Action (IA) models, were employed as the first tier models. As the higher tier models, Partial Least Squares–based Integrated Addition Model (PLS-IAM), and Quantitative Structure-Activity Relationship-based Two-Stage Prediction Model (QSAR-TSP) were used. CA and IA can either assume a target mixture is composed of similarly or dissimilarly acting chemicals, respectively. PLS-IAM and QSAR-TSP can consider both types of chemicals by using the toxicity data of similar mixtures and the structural information of chemicals, respectively. A case study was conducted to show ‘what does Eco-PDS do for the mixture safety assessment?’ This study highlights that Eco-PDS can be useful to easily screen the potential toxicity of mixture product by using different models simultaneously on the web; and to check which components are critical to the mixture toxicity in order to be safer products in time- and cost-saving methods.

TH147
Risk assessment of florists’ exposure to insecticide residues during normal professional tasks
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Flowers receive heavy pesticide applications prior to shipment to control various pests, insect infestation and disease carriers, such as mosquitoes, ticks, which can damage production and marketability. In order to evaluate the risk for florists exposed to insecticide residues during normal professional tasks, cotton gloves distributed to 20 florists (two pairs to each florist) were worn during two consecutive half days during normal professional tasks (from min 2 hours to max 3 hours/day) to measure their potential dermal exposure (PDE). The residual pesticide deposits on gloves samples were analyzed with a combination of gas and liquid chromatography and a multi-residue (QuEChERS) method. A total of 55 insecticides were detected on cotton gloves. An average of 6.65mg/kg insecticide residue per glove sample were measured. The active substances detected are known for their toxicological properties (acute toxicity, with an action on the nervous system). Many of them may affect the skin of the florists after dermal exposure and 3 of 55 are suspected of causing cancer after prolonged or repeated exposure. Novaluron are the substance active for which the highest average concentration (3.38 mg/kg). Clofentezine was both the active substance for which the highest maximum concentration (18.37 mg/kg), the most detected insecticide and the substance active has a PDE the most critical. Consequently, florists who worked for several years and handle a large number of flowers contaminated with insecticide residues, are exposed daily with a potential effect on their health.

TH148
Human health risk assessment of POPs in agricultural soils from Turkey
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In parallel to the improvement of agricultural and industrial activities, use of chemicals has increased throughout the world. Among these chemicals, persistent organic pollutants (POPs) are a group that are toxic, persist in the environment for long time, bioaccumulative and undergo long range transport. Although they have been banned under the Stockholm Convention, POPs are of particular concern to public health and environment due to their persistence and adverse health effects on the living organisms. Among POPs, organochlorine pesticides (OCPs), were widely used for agricultural purposes in Turkey similar to other regions of the world. Thus, they are present in environmental matrices as residuals from the past. In this study, OCPs were measured in soil samples from an agricultural area in Turkey. Additionally, carcinogenic and non-carcinogenic human health risks were evaluated from ingestion, inhalation and dermal exposure of soils containing these chemicals. Total $?_{28}$OCPs concentrations in soil samples were in the range of 0.080-4.15 $ng.g^{-1}$ dw. The hazard indexes (sum of calculated hazard quotients) for all samples were lower than 1 showing no significant risk of non-carcinogenic health effect. In addition, results of the carcinogenic evaluation showed that all calculated risk values were smaller than the reference value of $1x10^{-6}$ indicating no cancer risk for these chemicals in the region. The risk for children is about two times higher which is not surprising since body weight of children is lower and

ingestion rate is higher compared to adults. For non-carcinogenic risk the prominent exposure pathway was ingestion followed by dermal. For cancer risk, the order was ingestion>dermal>inhalation. In conclusion, OCPs from the ingestion, dermal exposure and inhalation of soil might not introduce a potential health risk to the people living in the area. However, dietary intake and other exposure pathways such as inhalation of air should be evaluated to assess overall health effect.

TH149

Derivation of Novel Uncertainty Factors for Health Risk Assessment: Application to Cleaning Product Ingredients

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Uncertainty factors (UFs) are commonly used by risk assessors to complement incomplete knowledge and inherent uncertainties, including extrapolations among mammals. In human and other mammalian studies, default values (e.g., 10-fold) are routinely used for intra- and interspecies variability. In fact, UFs are common in regulatory guidelines and hazard and risk assessment practice. Whether default UFs are sufficient for various chemical uses or specific chemical classes remains understudied, particularly for ingredients in cleaning products. Therefore, we systematically examined publically available acute median lethal dose (LD50), and reproductive/developmental no-observed-adverse-effect level (NOAEL) and lowest-observed-adverse-effect level (LOAEL) values for the rat model (oral) from the Cleaning Product Ingredient Safety Ini-tiative database. We employed a probabilistic chemical toxicity distribution (CTD) approach to identify likelihoods of encountering acute, subacute, subchronic and chronic toxicity thresholds for specific chemical categories and all available ingredients in cleaning products. For each CTD, threshold levels (95% CIs) were identified at 1^{st}, 5^{th}, 10^{th}, 50^{th}, 90^{th}, 95^{th}, and 99^{th} percentiles by the log-normal model. We then specifically identified UFs for: 1) acute (LD50s)-to-chronic (reproductive NOAELs) ratios (ACRs), 2) acute (LD50s)-to-chronic (developmental NOAELs) ratios (ACRs), 3) LOAEL-to-NOAEL ratios considering subacute/acute developmental responses. These ratios (95% CIs) were calculated from the pairwise threshold levels at different percentiles by using Monte Carlo techniques to identify UFs for all ingredients in cleaning products. Based on data availability, chemical category-specific UFs were also identified for Inorganic Acids and Salts and Aliphatic Alcohols, respectively. Our novel approach for deriving mammalian UFs represents a robust alternative to application of default values for ingredients in cleaning products and other chemical classes. This approach can also support data dossier development (e.g., read across) and screening-level hazard assessment when toxicity data is unavailable for specific chemicals.

TH150

High Throughput Development of No Observable Adverse Effects Levels for Use in Screening Level Human Health Risk Assessment of Cleaning Product Ingredients

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The American Cleaning Institute (ACI) is the industry trade association that represents the formulators of more than 90% of the consumer cleaning products in the United States. Recently, ACI set out to define and publish the universe of ingredients used in its members' products with the goal of identifying the publicly available hazard data for every ingredient and developing a screening level risk assessment. Using a tiered process, a complete screening information data set (SIDS) was sought for almost 600 cleaning product ingredients in relevant chemical groupings. Over 7,000 pieces of hazard data were compiled from previous EPA's HPVIS, OECD's HPV chemical archive, ECHA REACH dossiers, FIFRA Inerts Database, TOXNET and HSDB, EPA's Integrated Risk Information System, previous ACI risk assessments, HERA project risk assessments, CIR assessments, JECFA, and FDA SCOGS. Endpoints of interest included acute and repeated dose studies (oral, dermal, and inhalation), skin and eye irritation, toxicity to development and reproduction, mutagenicity, and carcinogenicity. Where data were not available for a specific ingredient, data gaps were addressed using grouping, read-across, and category approaches. A hierarchical approach was developed for deriving conservative No Observable Adverse Effects Levels (NOAELs) for use in risk assessment. Priority was given to ingredient-specific data over read-across data; to data from authoritative sources with a documented, defensible, and transparent methodology applied across data from multiple studies as opposed to data from single studies; to no or low effect levels from chronic, repeated dose, or development and reproductive endpoint studies as opposed to LD_{50} data from acute studies; and data with a Klimisch reliability rating of 1 or 2. Where data from defensible sources or data from chronic NOAEL studies were unavailable, assessment factors were applied to address uncertainties associated with test animal, test duration, and dose descriptor via conservatism. In some cases, oral NOAELs were used as surrogates for inhalation or dermal NOAELs. Where quantitative values were unavailable for either ingredient-specific or read-across NOAEL derivation, qualitative assessments of hazard such as FDA GRAS findings were utilized. The resulting database provides quantitative NOAELs for the majority of ingredients. The presentation reviews the number of ingredients for which NOAELs were developed from each source.

TH151

A critical overview of the mixture ecotoxicity data with pharmaceuticals and personal care products reported in the last 16 years

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Establishing regulations for the environmental risk assessment (ERA) of mixtures of pharmaceuticals and personal care products (PPCP) is an urgent need, since mixtures and not isolated substances are usually present in environmental compartments. In order to contribute to this challenging task, we aimed to present an updated state of knowledge regarding the design of the experiments and the interpretation of the results from the analysis of 53 articles published from 2000-2016, comprising 164 ecotoxicological data with PPCP mixtures. Results showed a clearly predominance of antimicrobials among the components usually evaluated as a PPCP mixture, representing almost 36% of the retrieved data. On the other hand, hormones and metabolite/transformation products were evaluated in only 1.2 and 0.2% of the evaluated mixtures respectively. 72.6% of the retrieved data involved the analysis of binary mixtures, while more complex mixtures covering ten or more components represented only 5% of the data. Acute assays largely predominated, while the chronic ones and those aiming to evaluate sub-lethal endpoints represented only around 30% of the total data. Regarding the experimental designs, 14 different strategies were identified in the retrieved studies. The fixed ratio design was employed for obtaining over 50% of the analyzed data, while multiple ratio designs were used in only 27% of them. In addition, the reasons for the choice of the concentrations of the mixture components were not justified in 4% of the studies. Concerning the approaches used to predict/assess the joint effects of components of PPCP mixtures, besides the two classical Concentration Addition (CA) and Independent Action (IA) models, 12 other approaches, including other models, graphics and indices used for this same purpose were identified from the retrieved data. A lack of consensus was observed concerning the use of the terms synergism/antagonism. In 1.2% of the retrieved mixture data, the term synergism was used to describe the possible interaction among the mixture components without explicitly referring to any reference model and without using a statistical method to compare the individual pharmaceutical and the mixture effects. We concluded that further advances in establishing regulations for PPCP mixtures of environmental concern depends on a priority establishment of consistent and uniform criteria for designing the mixture experiments and interpreting the outcomes.

TH152

First screening for (eco)toxicity testing of multi-component metal oxide nanomaterials

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Li-phosphate (LFP) and lithium nickel manganese cobalt oxide (NMC) are commonly used as cathode materials for printed batteries (electrochemical ink formulation). Recently, the use in their nanosized forms is gaining interest due to improved properties. These materials could end up in the environment at the battery end of life. Although the generation of data on toxicity of nanomaterials has increased in the last years, little attention has been given to multi-component nanomaterials. In this study, as a first screen for the toxicological characteristics of LFP and NMC, we evaluated their in vitro cytotoxicity on human cells and the toxicity to *Daphnia magna* as a model freshwater invertebrate species. LFP and NMC were tested at concentrations in the range of 62.5-1000 mg/L. Acute studies (48-hours) with *Daphnia magna* showed an EC_{50} of 500 mg/L and 700 mg/L for LFP and NMC, respectively. *In vitro* studies with A549 cells showed EC_{50} values of 1 mg/mL and 0.05 mg/mL for LFP and NMC respectively. Similar values were observed in Caco-2 cells: EC_{50} values of 0.5 mg/mL and 0.09 mg/mL for LFP and NMC respectively. Metal exposure concentrations in the test media were measured by ICP/MS after acid digestion to provide information on the total concentration. In addition, ultrafiltrated samples were analysed to provide information on the dissolved ion fraction. Around 10% of the lithium content dissolved into the two test media over 24 hours, whereas the dissolution rate of Co, Mn, Fe, and Ni was much lower (< 2%). In order to investigate if the effects observed were due to the dissolved toxic ions, ultrafiltrated test media was used in additional toxicity studies. Acute studies (48-hours) in *Daphnia magna* showed no effect. Similarly, *in vitro* studies with A549 and Caco-2 cells showed no effect for LFP and slightly effect for NMC. This study indicated that the exposure to the metals dissolved in the test media could not explain the observed effect after exposure to these nanopodwers, indicating a specific impact of the particulate fraction. Optical images demonstrated the accumulation of these particles in the carapace exterior and the digestive tract of *D. magna* and in inside cells (apparently not in the nucleus). Local release of toxic ions could still be responsible for the observed toxicity and deserve further investigations. Key words: multi-component substances, nanomaterials, ecotoxicity, *in vitro* test *Acknowledgments.* This study has been supported by BASMATI Project, ID: 616459 (2015-2018) under the H2020 Programme of the

European Commission

Human health: linking environmental exposure and human biomonitoring data for human health risk assessment of chemicals (P)

TH153

Modelling Pb blood concentrations in pre-school children living in a residential setting using MERLIN-Expo

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We report on model simulations performed using the newly developed exposure tool, MERLIN-Expo, in order to assess lead (Pb) exposure to pre-school children resulting from past emissions by non-ferrous smelters in Belgium (Northern Campine area). Exposure scenarios were constructed to estimate internal Pb exposure in children living in the vicinity of the former industrial sites as compared to children living in adjacent areas and a reference area. Model simulations at individual level are compared with biomonitoring data from a Belgian monitoring campaign (Flemish Government, 2008; Van Holderbeke et al., 2008) in order to verify the model performance of MERLIN-Expo when simulating complex scenarios that account for subject mobility, i.e., duration at the different considered locations (both indoors and outdoors) with varying exposure levels, and individual food consumption patterns. Adopting an assessment approach at individual level improves on more commonly performed generic exposure assessments at population level, by including intake of Pb from local and purchased food products, and taking into account the mobility of the participants, which results in a more comprehensive assessment of individual recent intake of lead. Also, the accuracy of the model predictions as compared to Pb biomonitoring data has been tested via different accuracy approaches and metrics. We calculated a Probability of detection (or true positive rate) of 0.93, reflecting that almost all blood levels above a chosen trigger value of 2.25 $mg.L^{-1}$ are predicted as such by the model; a Bias of 1.87, i.e. reflecting a slight overestimation of the model, and F1 and F2 scores of 0.65 and 0.8, respectively, confirming that most of the measured positives are detected as such by the model (high F2 score) with a slight overestimation (lower F1 score). Although the model predictions for individual children slightly overpredict the biomonitoring data, they overlap with the prediction interval calculated by MERLIN-Expo based on population averages (Fierens et al., 2016). Modelling exposure at individual level allows risk assessors to attribute the relative contribution of different exposure routes at various locations, reconstructing the exposure history for each individual in a complex site-specific exposure scenario. The slight overprediction of the actual observed Pb blood levels with a factor 2 is compatible with conservative decision-making.

TH154

Linking biomonitoring data to human health risk assessment for lead exposure-associate anemia risk among factory workers

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Lead-exposed workers may suffer adverse health effects under regulated blood lead (BPb) levels, currently. However, a probabilistic assessment about lead exposure-associated anemia risk is lacking. This study goal was to examine the association between lead exposure and anemia risk among factory workers in Taiwan. We first collated biomonitoring BPb and indicators of hematopoietic function data via health examination records. Benchmark dose (BMD) approach was applied to estimate the critical effect doses for detection of abnormal indicators. A risk-based probabilistic model was used to characterize the potential hazard of lead poisoning for job-specific workers by hazard index (HI). We applied Bayesian decision analysis to determine whether BMD can be implicated as a suitable BPb standard. Our results indicated that HI for total lead-exposed workers was 0.78 (95% confidence interval: 0.50 − 1.26) with risk occurrence probability of 11.1%. The abnormal risk of anemia indicators for male and female workers can be reduced, respectively, to 67 – 77% and 86 – 95% by adopting the suggested BPb standards of 25 and 15 $\mu g\ dL^{-1}$. We conclude that long-term exposure to lead in the workplace was significantly associate with anemia risk. This study suggests that current BPb standard needs to be better understood for the application of lead-exposed worker protection in different scenarios to provide a novel standard for health management.

TH155

Human exposure and risks of brominated flame retardants due to their elevated levels in dust and air in three types of indoor environment

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Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) are flame retardant compounds widely used in the furniture and electronics, construction materials and others. Their highest levels are found in the indoor environment due to the presence of the aforementioned materials inside the buildings and vehicles. Although their use has been banned or restricted, human exposure to these compounds is still ongoing due to the use of the materials containing flame retardants in the indoor environment where people spend 90% of their life-time. The monitoring, exposure and toxicity assessment of PBDEs and HBCDs are crucial for the human risk assessment refinement. This study aimed to measure the levels of PBDEs and HBCD in air and dust in three types of indoor environment (2 lecture rooms, 3 houses and 3 offices) and to assess the human exposure and risks due to these compounds. Two kinds of samplers were used concurrently: passive samplers containing the polyurethane foam sorbent (PUF) and low-volume active sampler with quartz filter for dust and particle phase collection and with polyurethane foam (PUF) for gas phase collection. Concurrently, the carbon dioxide levels, particle size distribution, air relative humidity and temperature were measured in each room. Also, surface dust was sampled using a vacuum cleaner at the end of the sampling campaign. After the sampling, the sorbents were extracted using non-polar solvent, concentrated and analyzed using GC/HRMS. The risk assessment was carried out with the aim of comparing contribution of different exposure routes and pathways. Human exposure via inhalation, ingestion and dermal route is occurring due to the presence of elevated levels of PBDEs and HBCDs in the indoor environment. Due to their potential of bioaccumulation, toxic effects, and persistence, their potential of negative effects on human health is likely. This work was financially supported by the Czech Science Foundation (CSF grant No. 14-27941-S) National Sustainability Programme of the Czech Ministry of Education, Youth and Sports (LO1214) and the RECETOX research infrastructure (LM2015051).

TH156

Phthalates in floor dust and exposure of children based on urinary phthalates metabolites

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Because of their ubiquitous application in consumer products, phthalates are widely present in the indoor environment. In this study, we measured levels and profiles of 16 kinds of phthalates in floor dust samples collected from house (n=112), four daycare center, and four workplaces. For some children (n=52), the first-void urine samples were also collected when dust was sampled. Six phthalates were detected in >70% of dust samples. Higher-molecular-weight phthalates, e.g., di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DnBP), diisononyl phthalate (DiNP), and diisononyl phthalate (DiNP), were detected at higher concentrations. DEHP occupied most significant composition, i.e., 53, 66, and 20% of sum of four major phthalates in dust of house, workplace, and daycare center, respectively. Meanwhile, the contribution of DiNP were 29, 26, and 74% for house, workplace, and daycare center, respectively. DiNP levels in dust were higher with more monthly income. Shorter ventilation time per week, and use of house deodorant/air freshener/insecticide spray were significant determinants of higher DEHP and DiNP concentrations in house dusts. For 52 children, phthalate ingestion rate via dust was calculated, then compared with total daily intake (TDI) estimated based on the urinary metabolite concentrations of diethyl phthalate (DEP), diisobutyl phthalate (DiBP), DnBP, and DEHP. Median contributions (%) to the TDI were 0, 1, 43, and 25% for DEP, DiBP, DnBP, and DEHP, respectively. Considering no correlation between levels of dust and urine, and low representativeness of dust samples, scenario-based exposure assessment based on the dust monitoring has limitations. However, our study showed importance of dust monitoring for an exposure assessment of certain phthalates such as DnBP and DEHP, and necessity of further study for alternative chemical like DiNP and DiDP.

TH157

Endocrine disruptive potentials of indoor air and dust samples assessed with human cell models in vitro

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More than 80% of our time is spent indoors where we are exposed to a number of chemicals that are present there. Impact of mixtures of these chemicals on human health is mostly not known yet. Although those compounds can enter our body by different routes, the exposure via inhalation is one of the most important. Pollutants can be inhaled from both air and particulate phase. Over the last decade it has been reported that the compounds from indoor sources possess endocrine disruptive potential which can lead to adverse health effects (e. g. carcinogenesis, reduced fertility, etc.). To evaluate toxic potential of indoor pollutants, it is convenient to use *in vitro* methods that are relevant, reproducible and relatively cheap. In this study, three different indoor environments (households, offices and lecture rooms) and three matrices (air particulate matter, gas phase and dust) were sampled for chemical and toxicological analyses. Toxicological analyses were focused on endocrine disruptive potentials (anti-/estrogenicity, anti-/androgenicity, thyroid and dioxin-like activity) and cytotoxicity. To increase relevance of the results to human health only human cell lines were employed for the study. Endocrine disruptive potential was assessed using reporter gene assays andcytotoxicity was evaluated *in vitro* by combination of three viability assays on non-carcinogenic respiratory tract model. From preliminary results it can be concluded that pollutants with estrogenic, dioxin-like, thyroid and anti-androgenic activity were present in the gas phase. Similarly, the dioxin-like, thyroid and anti-estrogenic effects were detected in particulate phase of indoor air. As for the dust, estrogenicity and dioxin-like activity as well as significant cytotoxicity were observed in all samples. Our data clearly show that compounds with endocrine disruptive potential are present in all three studied matrices in household, lecture rooms or offices environment. Together with chemical analysis data, the results from bioassays are used to identify the key toxicants and fractions which have contributed the most to the detected toxic potentials. Conducted experiments also confirmed the utility of *in vitro* bioassays for the toxicity assessment of indoor air and dust pollution. This work was financially supported by the National Sustainability Programme of the Czech Ministry of Education, Youth and Sports (LO1214) and the RECETOX research infrastructure (LM2015051).

TH158

Risk assessment on aggregate exposure to pesticide of agricultural operator

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Agricultural operators could be exposed to a range of pesticides while handling pesticides during mixing/loading and pesticide application. The present study employed the harmonised Agricultural Operator Exposure Model (AOEM) to estimate daily exposure based on current application techniques, comprising low crop application using vehicle-mounted or vehicle-trailed boom sprayers (LCTM), low crop application using hand-held spray equipment directed downwind (LCHH), high crop application using vehicle-mounted or vehicle-trailed broadcast air-assisted sprayers (HCTM), and high crop application using hand-held spray equipment directed upwards (HCTM). By utilising pesticide application data funded by the European Food Safety Authority (EFSA) in view of performing environmental risk assessment for pesticides (EFSA, 2015), aggregate exposures via dermal and inhalation routes were predicted for individual operators from three MS that represent different regulatory zones, comprising the UK (Central), Lithuania (Northern), and Greece (Southern). Predicted exposures were compared to Agricultural Operator Exposure Levels (AOELs) obtained from the Pesticide Properties Database (PPDB, 2016) for individual active substances, where a risk index less than 1 (RI< 1) indicates that no adverse health effects can be expected. For the UK, ten professional agricultural operators working in arable and orchard systems were chosen for randomised exposure estimations. The results indicate that both systems had extensive pesticide applications between April and August 2013 and the risk estimates varied across individual operators. Generally, operators from the orchard system showed a slightly higher percentage of working days with RI greater than 1 (0.9%-22.2%) for at least one active substance compared to those from the arable system (0.0%-14.3%). This suggests the need for further assessment of the risks for professional agricultural operators and potentially increased requirements for personal protective equipment. The presentation will provide results for the UK systems as well as comparing estimates for exposure and risk across the three regulatory zones.

TH159

Integrated exposure assessment to PAHs arising from the use of petroleum substances and comparison with biomonitoring data

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Aim Exposures to polycyclic aromatic hydrocarbons (PAHs) are ubiquitous. There are many sources and routes by which human exposure to PAHs occurs. However, the contribution of petroleum substances (PS) to PAH exposures for the general population (i.e. non-professional) has not been widely characterised and therefore the impact is not fully established. In view of the potential for petroleum substances to be included in the different REACH processes (notably Evaluation and Authorisation), the aim of the project was to identify integrated multi-source, multi-route (MSMR) exposure model(s) suitable for characterising exposure to PAHs including those arising from the direct consumer use of petroleum substances, as well as those occurring indirectly. **Methods and results** In a first phase of the project, a selection of the most appropriate models was made. Hereto, a list of 24 'available integrated exposure tools' was compiled based on models screened within two recent projects on integrated exposure modelling (the 4-Fun project and the CEFIC LRI project TAGS). After a first screening of model relevance for PAHs, a systematic inventory of various model aspects of relevant models was made, such as 1) model purpose, 2) exposure pathways considered in the models, 3) model applicability domain, 4) model parameterisation, 5) exposure pathways aggregation method, 6) ease of model use, 7) capability to predict internal levels in the human body. As an outcome, two models, namely MerlinExpo (http://4funproject.eu) and INTEGRA (http://www.integra.cperi.certh.gr/), were selected as the most promising MSMR tools to assess PAH exposures arising from the use of petroleum substances. In a second phase of the proeject, MerlinExpo and INTEGRA have been applied to predict PAH exposure in six distinct consumer use and environmental exposure scenarios of petroleum substances, i.e. 1) filling up motor oil of a car; 2) filling up a diesel car, 3) local exposure around oil refineries, 4) cosmetics and personal care products, 5) playgrounds and sportfields, 6) background environmental exposure. The models have been fed with exposure factors and substance/scenario specific data, gathered from different sources. Levels of 1-hydroxypyrene in urine as predicted by the models and comparison with matching biomononitoring data of this compound will be presented.

TH160

Accumulative effects of polycyclic aromatic hydrocarbons (PAHs) environmental exposure on telomere length

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Exposure to polycyclic aromatic hydrocarbons (PAHs) is believed to be associated with many adverse health impacts, including lung cancer. During the exposure period, cell division is promoted for tissue renewal and function maintenance, which is reflected in the shortening of telomere length. The relationship between telomere length and environmental pollution has been investigated in various studies; however, the accumulative relationship between these factors over decades, which is the latent period of cancer development, has not been studied. Therefore, 137 pairs of lung adenocarcinoma patients and matched cancer-free controls who had been exposed to similar primary PAHs exposure were recruited in this study. Their exposure levels of PAHs environmental factors during the past three decades were assessed via questionnaire survey and their relative telomere length (RTL) was measured. It was found that the accumulative exposure of solid fuel usage in three decades was negatively correlated with the RTL ($p< 0.05$), reflecting in both of heating fuel and cooking fuel. And, education and occupation were related to both environmental exposure and the RTL ($p< 0.01$). Thus, it can be figured out that sociodemographic factors affect telomere length via correlations with environmental exposure. What's more, when the primary environmental exposure effect of PAHs was eliminated, the RTL was shorter in the lung cancer group than in the matched control group (p=0.02). In addition, the RTL was shorter at higher cancer stages ($p< 0.05$). Therefore, telomere length can be considered as a marker of lung adenocarcinoma risk and progression.

TH161

Biomonitoring of human exposure to benzene and toluene in oil extraction area of Muanda, Congo

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Oil extraction, crude oil transportation and oil processing are industrial activities that potentially could cause adverse effects on environment and human health. The coastline of the Democratic Republic of Congo is an oil extraction area, both off- and on- shore. Over the last few years, people form the coastal region of Muanda, supported by political representatives and non-governmental organizations, have expressed concerns about the effects of oil extraction from this region on the environment and the health of the population. In this context, air samples near extinct torch or a fuel storage place, revealed high concentrations of many volatile compounds, mainly aliphatic hydrocarbons, but also benzene and toluene in significant amounts. Thus, in order to evaluate the degree of human exposure to benzene and toluene, urine samples were collected from 53 individuals (34 adults and 19 children, 26 females and 27 males) in four different locations. Urinary concentrations of hippuric acid (as biomarker for toluene exposure), t,t-muconic acid and SPMA (as biomarker for benzene exposure) were determined. For hippuric acid we found median (range) concentrations of 84 mg/L (range: 50-401 mg/L) for Mangrove group; 213 mg/L (range: 50-763 mg/L) for "oil wells" group; 240 mg/L (range: 50 - 1249 mg/L) for "oil handling" group. The concentration of t,t-MA in urine was 0,05 mg/L (range: 0,05 -0,15 mg/L) in Mangrove group; 0,15 mg/L (range: 0,05 - 1,25 mg/L)oil wells group; 0,29 mg/L (range: 0,05 - 1,02 mg/L)oil handling group. The concentration of SPMA found in urine was 0,24 μg/L (range: 0,05 -1,58 (μg/L) in Mangrove group; 0,59 mg/L (range: 0,05 to 6,7 (μg/L)oil wells group; 0,51 μg/L (range: 0,11 - 6,85 μg/L)oil handling group. In conclusion, urinary biomonitoring had revealed that the 'oil wells' group and the 'oil handling' group have shown urinary concentrations slightly higher than the 'mangrove' group. Nevertheless, these analyses did not reveal significant evidence of increased risks for human health in relation to oil extraction activities in Congo.

TH162
A probabilistic risk assessment for the impact of airborne paraquat-associated exposures on Parkinson's disease
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BACKGROUND: Evidence showed that the herbicide paraquat (PQ) is significantly associated with the prevalence risks of Parkinson's disease (PD), the second most common neurodegenerative disease. **OBJECTIVE:** The primary objective of this study was to predict PQ-induced PD prevalence risks on the basis of projected rates of increase in PQ pollution and population levels. **METHODS:** Age-dependent and specific cumulative PQ doses were explored by employing a physiologically based pharmacokinetic modeling to predict bioaccumulations in brain of human beings. In dose-response analysis, a relationship of PQ concentrations and inhibition of dopaminergic cell viability was constructed by the Hill equation model. The PQ exposure-associated population attributable fraction for PD was derivedbased on an epidemiological study. Risks of PQ-induced PD were characterized probabilistically by assessing population excess risk and evaluating contribution of PQ use to PD prevalence. **RESULTS:** Under 50% risk probability, inhibitions on dopaminergic cell viability for people aged < 50, 50 – 59, 60 – 69, 70 – 79, and ≥ 80 yr are 27.4% (95% CI: 16.4 – 38.4), 48.4 (36.7 – 60.2), 49.7 (37.8 – 61.6), 50.4 (38.4 – 62.5), and 51.0 (38.9 – 63.1), respectively. In Taiwan, the largest PQ exposure contributions occurred in its positive trend during 2004 – 2011, with the PQ contributing nearly 21% and 24%, respectively, to the PD prevalence rate among age groups of 70 – 79 yr and ³ 80 yr. **CONCLUSIONS:** The time trends in prevalence and incidences of PD are embedded in a background relationship between PD risks and PQ exposures. It is necessary to improve insights into the perspective on influences of pesticide-associated exposures on neurodegenerative diseases. \n

TH163
Development and evolution of pesticides risks indicators to human health in Belgium and Wallonia
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Within the framework EU Directive 2009/128 of the European Commission, Members States have to develop national action plan for reducing the risk of plant protection product (PPP). National plan must contain quantitative objectives, measures and a calendar for implementation of the measures taken on the uses of PPP. In Belgium the Nationaal Actieplan/Plan d'Action National (NAPAN) is composed of federal and of regional plans, each containing specific and joined actions. As requested in the Wal.9 action of the Walloon Program for the Reduction of Pesticides (WPRP) predefined harmonized risk indicators have to be developed and calculated based on the statistics of uses of PPP to assess progress made by public authorities in terms of reducing risks and the effects of PPP for public health and professionals. To address this action Pesticides Risks Indicators (PRIs) were developed based on the chronic toxicity and the persistence of active substances and on the sales and on the uses of the molecules in Belgium and in the Walloon Region. These PRIs are calculated using the Toxicological Risks Indexes (TRIs) that take into account 3 chronic toxicological endpoints: carcinogenicity, neurotoxicity and reproduction and developmental toxicity plus endocrine disruption potency. As proposed in the Québec Pesticide Risk Indicator scores were attributed to pesticides based on the association between exposure and effects with a high score for well-established associations and a low score when no association has been made. Moreover persistence of the pesticide in the environment was also taken into account as higher persistence induces potentially higher exposure and therefore potentially more impacts on human health. Statistics on the sales and uses of PPP in Belgium and Wallonia were used to calculate PRIs by multiplying TRIs with quantities of PPP. The PRIs were calculated for 443 active substances in Belgium using sales of PPP for the years 1995, 2005 and 2010 to 2015 for professional and non-professional users. In Wallonia, the quantity of active substances used by farmers between 2010 and 2014 allowed calculating evolution of PRIs. The evolution of PRIs might allow the regional governments assessing the effectiveness of the measures taken within the WPRP for the reduction of risks associated with the use of PPP for public health.

TH164
Assessing the food safety risk in children exposed to organophosphate pesticides in Taiwan
M. Ling, Department of Food Science, National Taiwan Ocean University / Department of Food Science
Baby and adult populations have large differences in eating habits. Baby food cooking method usually simple, style varied less than adult, mostly from commercial baby food or cook by their own fresh fruits or vegetables. This may cause intake some specific food contaminants, for example: food additives and pesticide residue. Infants are the most vulnerable populations, even if a small number of contaminants in food may cause hazardous to health and resulting in a negative impact on health. A large amount of infants and children (1-6 years) food consumption data and body weight parameter were obtained from the 2005–2008 Nutrition and Health Survey in Taiwan (NAHSIT). Using total diet study (TDS) method to construct the food list for the Taiwan infant then to analyze food additives and pesticide residue concentrations in infant representative food items to conduct exposure and health risk assessment, establish the average daily intake of food additives and pesticide residues average daily dose (ADD) and acceptable daily intake (ADI). Evaluate hazard index (%ADI) of food additives and pesticide residues in exposure population. Then according to %ADI evaluation results, establish real-time interactive handheld mobile health systems, application human-machine interface technology, and set up the mobile applications. It will facilitate people to evaluate infant food safety risks and food contamination, providing a warning of high contribution food items to recommend and reduce food intake, and thus achieve the purpose of risk communication and health education. We analyzed 76 representative food samples with salicylic acid, benzoic acid, sorbic acid, dehydroacetic acid, and p-hydroxybenzoate, respectively. The results showed that only salicylic acid and p-hydroxybenzoic acid were detected, the human body no significant harm. We also analyzed 310 pesticide residues in 76 representative food samples. Ninety-nine pesticides were detected. We estimated the chronic health risk in infants and children with average food intake of the whole group. The risk was lower than 6.29 %ADI, which was acceptable risk.

TH165
Assessing BPA intake from canned food consumption based on web survey
Y. Li, P. Lu, Y. Luo, D. Luh, S. Chen, Chung Shan Medical University / Department of Public Health
The dietary intake is the major bisphenol A (BPA) exposure route in human that can cause BPA related adverse effect. The large-scale exposure risk of humans to BPA through dietary sources in Taiwan is less well studied. The aim of this study was to assess average daily dose (ADD) and hazardous quotient (HQ) of BPA exposure risk from canned food consumption in different age-sex groups in Taiwan. We reanalyzed the BPA concentrations from five canned food sources (meat, seafood, beans, fruits, vegetables, bereaves and juice) and ingestion rates to estimate the contribution of variances to ADDs and potential humane health effect in different age-sex groups. Total sample size was 1136 with 97.8% response rate. This study found that the highest BPA ADD was found in ≥ 65 years age group (0.334 and 0.398 μg/BW-kg/day for male and female), whereas the lowest BPA ADD were 20-64 years age group (0.143 μg/BW-kg/day for female) and 0-19 years age group (0.177 μg/BW-kg/day for male). Otherwise, all estimated HQs of BPA intake by age and gender were less than 1. Results indicated that there was no hazard risk among all age groups via the dietary exposure from canned food. Based on EFSA guidelines, HQ of BPA intake in ≥ 65 years have a higher potential to pose risks through the dietary intakes than other age groups. Therefore, a combination of multiple exposure routes and the long-term exposure in specific population should be concern in the future.

TH166
Toxicological properties of PM10: relationships with markers for wood burning and traffic and association with health biomarkers in an adolescent population of Flanders
R. Van Den Heuvel, VITO NV / Environmental Risk and Health; G. Koppen, A. Colles, E. Govarts, VITO / Environmental Risk and Health; J. Staelens, VMM; M. Mampaey, LNE; G. Schoeters, VITO / Environmental Risk and Health
The study commissioned by VMM within the framework of JOAQUIN investigated the toxicity of particulate matter (PM_{10}) collected in an urban area (Antwerp-Borgerhout) and a background site (Houtem). The study commissioned by the Flemish Government and within the framework of the Flemish Centre of Expertise on Environment and Health (2012-2015) investigated the toxicity of PM_{10} collected at an industrial site (Zelzate) and a background location (Houtem). The sampling campaigns started on April 1, 2013 and ended on May 8, 2014. The studies aimed (1) to measure different cellular responses (cytotoxicity, pro-inflammatory changes, DNA damage) of airway epithelial cells to PM_{10} and mutagenic and oxidative potential of PM_{10}; (2) to verify spatial differences in the responses; (3) to identify pollutants which are associated with the observed biological effects; and (4) to investigate whether human biomarkers from the biomonitoring campaign in adolescents living in the industrial site were related to in vitro results. The PM_{10} fraction induced a concentration-dependent decrease in cell viability and an increase in inflammatory cytokine induction (il-8). Compared to the background location a significant increase in inflammatory mutagenic and oxidative potential of PM_{10} collected in the urban area was seen. Compared to the background location a significant increase in mutagenic and oxidative potential of PM_{10} collected in the industrial location was observed. The results showed that both PM mass as well as the chemical composition of PM were determinants of the observed biological effects. Metals in PM_{10} played an important role in cytotoxicity, pro-inflammatory cytokine induction, oxidative potential and oxidative DNA damage. Markers for wood burning were associated with increased cytotoxicity and mutagenic responses. BC and EC correlated with cytotoxicity and with the

inflammatory, mutagenic and oxidative potential of PM_{10}. In addition, toxic properties of the particles were associated with biomarkers measured in biological samples of adolescents living in the study area. An increase in the oxidative potential of PM_{10} samples induced an increase in the production of il-1β in breath condensate of the adolescents (reflecting more inflammation) and more DNA breaks in blood of the adolescents as measured by the comet assay in blood samples. The pH of the breath condensate decreased (reflecting more inflammation) as the inflammatory potential of PM_{10} samples increased.

TH167
SETAC Human Health Interest Group
B. Mulhearn, Ensafe Inc.

Improving the environmental assessment of complex composition substances and mixtures for Chemicals Management (P)

TH168
Assessment of SID of inorganic UVCBs in recycling industry
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Inorganic UVCBs in the metal sector are typically by-products or metal rich intermediates obtained during metals extraction. These substances are produced starting from primary and/or secondary/recycled source materials (e.g. slags, slimes, flue dusts, drosses or doré blister, anode, bullion, matte..). From a regulatory point of view, inorganic UVCBs are subject to REACH Regulation, as any other substance manufactured or imported in Europe above 1 ton per year. Meeting REACH requirements when registering UVCBs is a highly demanding exercise especially when dealing with substance identity. The main peculiarity of an inorganic UVCB is its spatial and temporal variability, even at production site. Due to the importance of the metals content in industrial streams, elemental analyses are regularly performed and elemental data are available, demonstrating varying concentrations of the metal constituents in the UVCB. On the other hand, the speciation information (i.e. definition of which chemical form metals are present in the UVCB) is often unknown, due to limitations of available analytical techniques. Industry is working on a pragmatic approach to meet regulatory requirements. An ad hoc template was developed to structure substance identity information following a REACH alike approach: the template lists process, source and composition as the key parameters. Starting from these, industry assessed the parameters to identify which could be considered decisive and which will only be indicative in assessing substance identity and substance sameness. In practice, UVCB substance identification is based on identification and reporting of decisive parameters (for fixed depictants, or with low variability) and on parameters that are only indicative (i.e. with medium variability) and parameters with low substance identification potential (i.e. with large variability). Complying with regulation while remaining competitive in the European market and the circular economy objectives represents a big challenge for the metals industry.

TH169
CRANCS2: promotion of a new methodology to deals with Natural Complex Substances type 2
P. Thomas, CEHTRA SAS; P. Bicherel, KREATiS
NCS type 2 are Natural Complex Substances (NCS) whose less than 90% of the composition is known. As well as being volatile and hydrophobic like the other NCS, they often are difficult material to manipulate in laboratories (resins, gums, cires, *etc*.). Current methodologies which have already been performed (sonification and heating) may denature the substance. Moreover lack of information regarding their composition do not allow to apply alternative methods, QSAR models or constituents approach for instance. Therefore analytically identification of the constituent is still required to predict physicochemical and (eco)toxicological properties of substances. The methodology presented is a block approach where NCS2 is divided into 3 parts in order to characterise the composition and the aquatic toxicity of the substance. The first block corresponds to terpenes fraction. Their constituents highly volatile can easily be characterised by GC. The second block is relative to more soluble constituents which can be terpene acids and aromatic acids, but also phenols and flavonoids. These component can be used identified via LC-MS. The third block is assumed to be inert. It corresponds to the resin, tannins, wax *etc*. with high molecular weight, insoluble, so non-bioavailable to aquatic organisms. The project aims to isolate and characterise the three parts of SNC2, then to compare the aquatic toxicity of global NCS2 dissolve into solvent with the respective toxicity of each block. In parallel, a calculation method already developed for WAF toxicity is applied in order to anticipate the aquatic toxicity of the different blocks.

TH170
UVCB substances: Methodology for substance identification and application to fate and hazard assessment
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Keywords: UVCB, hazard assessment, G SMILES UVCB substances are commonly described by generic substance identifiers such as names of chemical classes, structural formulas, reaction steps, physicochemical properties, or spectral data. A methodology allowing structural description of UVCB substances has been developed. Defining an inambigious structural description aims to complete the following two tasks: to allow performing of *in silico* hazard assesment, where structutal information is essential, and to contribute towards fulfilling the requirements of implementaion of any chemical related legislation. It is also envision that the methodology could be used as a tool for the identification of the so-called drivers, i.e. those constituents or classes of constituents which are responsible for eliciting a given toxic effect atributed to the UVCB as a whole. The methodology, based on the generic substance identifiers, allows coding, generation, and selection of representative constituents. Two main formats are developed: Generic Simplified Molecular-Input Line-Entry System (G SMILES) and Generic Graph (G GRAPH). G SMILES is a SMILES-based single line notation coding fixed and variable structural features of UVCBs. G GRAPH allows generation of constituents coded in G SMILES, calculation of physicochemical properties, simulation of reactions, selection of constituents and their storage. There are two main types of selection of constituents. The first one is an end-point driven selection based on ranges of a characteristic property, e.g. log *Kow* values are critical when assessing bioaccumulation potential. The second approach is a statistical procedure for random selection of a representative sample from a population of constituents. The representative sample is the minimum sample size of randomly chosen constituents that at an agreed confidence level and tolerated error reflects the population. It means that the proportion of characteristics of the objects in the population will be preserved in the representative sample. The procedure was validated both theoretically and on a real case study and then applyed to Ames mutagenicity assessment of a petroleum UVCB substance.

TH171
Practical Considerations for PBT Assessment of Complex Mixtures with Difficult Properties
D. Carson, Blue Frog Scientifiic Limited; T. O'brien, M.C. Jaimes, Ecolab; K. Paul, Blue Frog Scientific Limited / SLS
REACH requires that a PBT assessment is conducted for all substances manufactured or imported in quantities of >10 tonnes per year. Conducting a PBT assessment for complex substances (multi-constituents & UVCBs) presents significant challenges under current ECHA Guidance, especially for the environmental elements of the assessment. These challenges can be further compounded when some or all the constituents of a complex substance have properties which make the practical assessment of persistence, bioaccumulation and toxicity very difficult. We present a case study of a registered UVCB consisting of non-homologous constituents, with varying degrees of solubility, that are known to be surface active. ECHA recently issued a formal request for a comprehensive range of environmental studies to support the PBT/vPvB assessment, which should focus on all constituents >0.1 % (w/w). Such a request is to some extent consistent with ECHA Guidance, however there is currently no standardised approach to deal with such a specific situation offered by ECHA or other recognised guidance documents. We present and discuss the merits and weaknesses of a more proportional and tiered approach to the PBT assessment difficulties faced for this type of substance, with the purpose of developing an integrated strategy for testing the relevant components of UVCB substances with difficult properties. The integrated approach includes a sequential screening process for relevant constituents, followed by targeted use of higher-tier environmental fate and ecotoxicity studies, in support of an overall assessment of PBT for the substance. Particular attention is paid to the following: Grouping of homologous constituents for targeted laboratory testing Screening of key physico-chemical properties for representative substances within each group Screening for potential persistence of representative substances within each group followed by targeted simulation testing Screening for potential bioaccumulation of representative substances within each group, followed by targeted vertebrate testing as a last resort Approaches to chronic ecotoxicity testing considering bulk UVCB versus constituent testing approach A further intention of this presentation is to stimulate discussion and correspondence between industry and the authorities, to work towards developing a robust and integrated approach to this difficult aspect of hazard assessment under EU chemical control legislation.

TH172
Do natural plant extract substances, such as Essential oils, PBT candidates ?
R. SAMSERA, C. Durou, P. Thomas, CEHTRA SAS
Under the REACH registration program several kinds of substances are considered: monoconstituents, multiconstituents, & UVCBs. Across these substance (as such mixtures are named under REACH) types several families consistently present a challenge for data acquisition, especially to hazard property testing for ecotoxicology and environment. Many fragrances fall into one of these challenging groups. \n Fragrance chemicals can be of natural origin or synthetic and one particular group of natural fragrances that falls under the title of

multiconstituent/UVCB, the Essential oils are amongst the most difficult to assess:. Essential oils (EO) are natural complex mixtures (NCS), composition knowledge is variable (from less than 2% to more than 90%), liquid to solid/resin [1], containing multiple constituents (up to several hundred) with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. In order to acquire the data required for registration of these substances, several testing strategies have been produced based on available guidance document (CLP, R7a,b,c, R11, EFEO/IFRA guidance [2]). We will present that whatever the testing scenario, if you follow the advice provided in the available guidance documents, then the conclusion will always be the same: potentially P & potentially B. We will also discuss some reasonable solutions to avoid some inappropriate conclusions.

TH173

Assessing the Bioconcentration Potential of Fragrance Ingredients in Fish via Dietary Exposure with Internal Chemical Benchmarking

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Essential oils are fragrance materials that are registered as natural complex substances (NCS) under the European REACH legislation. One of the categories of information required in a REACH registration is information about the potential for bioaccumulation of NCS by fish. Determining the bioconcentration factor (BCF) of essential oils cannot be readily accomplished using a standard flow-through uptake/depuration experiment (e.g., the previous OECD 305 protocol), because it is difficult to maintain constant and defined concentrations of the substance in water during exposure. The updated OECD 305 guideline offers a dietary exposure approach which requires 7-14 days' uptake phase plus up to 28 day's depuration. Previous studies documented that the benchmarking technique can reduce the length of the aqueous exposure experiment and also correct for the effect of growth dilution. Here, we evaluated an abbreviated BCF-determination methodology with a mixture of test and benchmarking compounds (including α-pinene, camphene, limonene, 2-t-butylcyclohexyl acetate (Verdox), cashmeran (DPMI), cyclohexyl salicylate, acetyl cedrene, musk xylene, globanone and decamethylcyclopentasiloxane - D5, hexachlorobenzene - HCB and 2,2´,5,5´-tetrachlorobiphenyl - PCB52) via single contaminated dietary exposure and with benchmarking technique. Results show that the concentation of chemicals in the fish followed the first order kinetic model with k_T ranging from 0.0220 d^{-1} (musk xylene) to 0.5999 d^{-1} (Verdox). The BCF values derived from the calculated uptake rate constant (k_1) using the Arnot & Gobas model divided by the k_T for the tested chemicals and were in the range of 230 (Verdox) – 6300 (musk xylene) L kg^{-1}. The benmarked BCF (BCF_{BM}) ranged from 240 Lkg^{-1} (Verdox) to 7500 L kg^{-1} (musk xylene). These BCF data agreed well with the prevous ones from other studies, suggesting that our proposed method is working well. We concluded that it is feasible to measure the BCF in fish by exposure to a single oral dose of multiple test and benchmarking chemical(s). This method will be further applied to real essential oils (undergoing at the moment) to measure the BCF of compositions by fish and to improve the risk assessment of NCS such as essential oils. The results from experiment with real essential oil will be presented at the conference.

TH174

Determination of PNEC values for risk assessment of natural complex mixtures: options and limits

C. Durou, R. SAMSERA, P. Thomas, CEHTRA SAS

Under the REACH registration program several kinds of substances are considered: monoconstituents, multiconstituents & UVCBs. Across these substance (as such mixtures are named under REACH) types several families consistently present a challenge for data acquisition, especially to hazard property testing for ecotoxicology and environment. Many fragrances fall into one of these challenging groups. Fragrance chemicals can be of natural origin or synthetic and one particular group of natural fragrances that falls under the title of multiconstituent/UVCB, the Essential oils are amongst the most difficult to assess. Essential oils (EO) are natural complex mixtures (NCS), composition knowledge is variable (from less than 2% to more than 90%), liquid to solid/resin [1], containing multiple constituents (up to several hundred) with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. REACH requires Environmental Risk Assessment (ERA) to be performed at a tonnage level of 10 tonnes or more per year. ERA involves several physico-chemical property values to calculate environmental distribution and PEC values as well as ecotoxicity values to derive a PNEC. Several guidance documents are available to perform such calculations. Regarding the mixture approach, CLP guidance states that the PNEC of a mixture should be based on the PNEC of a Lead Substance Indicator (LSI) as opposed to the LSI PEC value. How is this applicable if nearly no information is available on the substance composition? The acute toxicity data can be generated using WAF approach but the outcomes cannot be used to derive a PNEC for other compartments than the aquatic compartment. Some say that a PNEC based on the WAF approach is not conservative enough while it is not possible to actually monitor what happens in the environment. But are PNEC WAFs actually less conservative than PNEC LSI (regarding composition knowledge and available dataset for LSI substance?), and is PNEC LSI a worst case representative of a mixture in the environment? This poster will present different options for data generation and discuss PNEC calculations based on LSI approach and WAF approach and compare the results.

TH175

Evaluating uncertainties in the risk assessment framework for petroleum hydrocarbons

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Risk assessments (RA) evaluate exposure, relative to conservative chronic effect-based hazard thresholds. Variability in the exposure and hazard sides of the assessments introduce uncertainties into the RA, which can cause concern on how to appropriately manage risk under chemicals management programs. The RA framework used for petroleum substances, e.g., the hydrocarbon block method (HBM) as used in PETRORISK, has evaluated the impact of these uncertainties by compiling compositional, effects, and monitoring data for a wide variety of scenarios and substance types. These data have characterized the variability that is introduced into the RA through uncertainties in the compositions, emissions, and chronic thresholds using probabilistic methods. For example, effect thresholds were based on a probabilistic analysis of a large database of toxicity data on a variety of species to account for variability in the sensitivity of the test species, but also the uncertainty in the estimated species sensitivity. Further, variability in the composition of the petroleum substances was evaluated by evaluating relative risks across a range of measured compositions within a particular petroleum substance category. Also, emissions estimates were compared to available monitoring data. The results of this analysis indicate conservative RA are obtained due mainly to conservative emissions estimates. Therefore, the HBM for risk assessment provides a frame of reference for evaluating the impact of uncertainties for other UVCBs where read across can be justified on the basis of physicochemical properties and mode of action.

TH176

Practical aspects in the aquatic toxicity testing of complex fragrance ingredients, synthetic or of natural origin (multi-constituents and UVCBs)

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Fragrances ingredients are relatively small molecules (MW generally < 300 g/mol), mostly liquids although some are solids or highly viscous resins. Their composition can vary from pure substances, to a mixture of known isomers or chemically different constituents (multi-constituents, MCs) or UVCBs (Unknown or Variable in Composition and/or Biological origin) such as natural essential oils or resinoids. Some constituents present physico-chemical properties that cause difficulties for aquatic tests, eg: solubility as low as the μg/l level; volatility up to 200 Pa, or they may be unstable in water depending on the pH. To overcome the inadequacies of standard guidelines, the OECD Guidance Document Number 23 for aquatic testing of difficult substances is often applied. Testing of fragrance ingredients often results in, total loss of substance at the end of some algae studies, difficulties maintaining concentrations just below the solubility limit, etc. Modifications proposed to the standard guidelines are often insufficient. Here we report examples of preparations of Water Accommodated Fractions (WAFs) for testing the toxicity of difficult fragrance ingredients on the acute immobilization test with *Daphnia* and/or algal growth inhibition; firstly on two synthetic MCs containing mostly volatile constituents, whereby at least one constituent has been measured analytically as a tracer. Some of the tests were conducted as confirmation in an effort to further validate the use of the predictions (iSafeRat predictions were also used to generate the LL/EL50's on fish, Daphnia and algae). Other WAF preparation regimes have also been used to test various UVCBs (natural essential oils and resins), whereby the organic material present at the start and end of each test was verified. A saturated solution of a hydrophobic reactive aldehyde (stirring and settling) was used to test the effects of the mixture of the parent and transformation products. Here both the total organic carbon and the parent compound (analytically measured by LCMS-MS as tracer) were determined during the duration of the tests; ecotoxicological results given as nominal loading rates. It is concluded that the approach for testing the aquatic toxicity of complex fragrance ingredients will depend on the composition, properties of the constituents, and may be unique to each specific case. The question of relevance of results will be discussed in the context of meaningful environmental hazard and risk assessments.

TH177

Test solution preparation for UVCB substances - a review of the water accommodated fraction methodology

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Aquatic toxicity testing required for hazard assessment of chemicals requires

consistent and measurable exposures to establish dose-response relationships. Preparing test solutions for mono-constituent substances, particularly if they are relatively stable and miscible in water, is generally straightforward. However, test media preparation for UVCBs (substances of Unknown or Variable composition, Complex reaction products or Biological materials), such as petroleum substances, presents considerable challenges as many standard approaches are not considered to be appropriate for these substances. Concawe – the European industry association for oil companies – developed the water accommodated fraction (WAF) methodology for ecotoxicological testing of petroleum products.[1] This has been established and internationally recognised in guidance documents as an accepted methodology for testing these products (OECD, IMO, ECHA, UN GHS). WAFs are prepared by gently stirring the test substance in the presence of aqueous test media until equilibrated. After a settling period the test solution is drawn from the base of the vessel to prevent disturbance of the surface layer. Care must be taken to minimise the formation of dispersed droplets in solution, as these can lead to physical effects and an overestimation of the exposure concentration. Chemical analysis should be performed to confirm consistent exposure concentrations although this is not always feasible due to constraints in available analytical techniques. In all cases WAF toxicity results should be expressed in the form of nominal loading rates, as the composition of test exposures are loading rate dependent and measured concentrations, when viewed in isolation, can be misleading. This poster will present an overview of the WAF as the standard methodology for preparing test solutions of poorly soluble UVCBs and incorporate recent experience and specific challenges associated with the WAF preparation plus the interpretation of the data generated. [1] CONCAWE (1993). Report 92/56: ecotoxicological testing of petroleum products.

TH179

Understanding WAF results used for the assessment of aquatic toxicity (CRANCS project)

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Nowadays, while it is generally recognised that ecotoxicity studies can be performed using the Water Accommodated Fraction (WAF) method (OECD, 2000) at least for Classification and Labelling purposes, there is some concern that results of such studies cannot be easily validated or interpreted. Their interpretation is often considered as ambiguous, and a workable method to transform the loading result to derive a meaningful PNEC (Predicted No Effect Concentration) for environmental risk assessment where it is compared to a PEC (Predicted Environmental Concentration) currently doesn't exist. Due to the high hydrophobicity of many constituents in Natural Complex Substances (NCS), the WAF method is used in the CRANCS project in order to maximise dissolution in the aqueous phase to reach a thermodynamic equilibrium. The results of the project have shown that: 1. The WAF method allows to successfully dissolve the constituents of a mixture to a stable equilibrium concentration. 2. Critically, the toxicity data obtained thanks to the WAF method can be interpreted depending on the concentration of the constituents into the accommodated fraction. Moreover, based on several ecotoxicological WAF studies, KREATiS has developed an iSafeRat® calculation method which can accurately predict the aquatic toxicity to fish, daphnid or algae where the additivity calculation method proposed by the CLP is much too severe, often unjustly classifying NCS as Acute 1 under GHS. Within the framework of CRANCS, different mixtures including an artificial, "designer mixture", have been studies in order to test the applicability of the WAF in risk assessment on one hand, and the applicability of the iSafeRat® calculation method on the other hand for its application for chronic toxicity. Ultimately the work accomplished in this project help to better understand the usefulness of the WAF approach and to strengthen its application in hazard assessment. The project itself should help to refine the risk assessment for NCS type 1 substances.

TH180

Matrix effects, bioavailability/bioaccessibility and the risk assessment of grease thickeners in situ in base oil

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In the grease manufacturing process, unique interactions (called matrix effects) occur which are more appropriately defined as physical bonding effects between the grease thickener and the base oil rather than a specific chemical reaction per se. It is proposed that the matrix effects have a significant impact on the bioavailability/ bioaccessibility of the grease thickener substances. The European REACH Grease Thickeners Consortium (ERGTC) have discussed approaches to considering the risk assessment of thickeners for their typical manufacture and use (*in situ* in a base oil), and how matrix effects from the formulation of greases should be reflected in the interpretation of the hazard data. The presentation reviews the use of additional 'non-standard' tests to build sufficient weight of evidence to assess the bioavailability/bioaccessibility of grease thickener substances when they are *in situ* in base oil. The additional tests which the ERGTC are currently preparing consist of a study to evaluate the leaching of the thickeners from base oil into the aquatic compartment and an everted gut study to evaluate the likelihood that the substances will be sufficiently bioaccessible that they will cross the gut wall. The ERGTC followed a 'generic approach' for substances previously registered under REACH; registering the thickeners in their isolated form as a worst-case review of the hazards of the thickeners themselves. However, where this was not appropriate, a 'modified approach' was used; for example, where a dataset is available on the isolated substance but everted gut and leaching studies are proposed to provide weight of evidence that additional long-term mammalian studies are not relevant. The ERGTC have proposed an 'alternative approach' for new substances based on the premise that the lack of bioaccessibility/bioavailability from the everted gut and leaching studies will scientifically justify that the hazard/risk is controlled and remove the need for certain *in vivo* studies. It is proposed that the data and strategy are used as the basis for sector specific guidance for evaluating and interpreting the hazard data for substances which occur *in situ* in base oil and how the data should be used to evaluate the associated risks. Acknowledgement - The authors thank the members of the ERGTC Technical Working Group for their assistance in developing the alternative approach.

TH181

Physicochemical and biological characteristics of leachate from biochar produced from malt spent rootlets

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The present study investigates the physicochemical composition of water aliquots derived from biochars produced from the pyrolysis of malt spent rootlets, in combination with the concomitant toxicological profile in each case. Specifically, physicochemical parameters and heavy metal ions were determined in aliquots of six serial washes of biochar (1.456 g of solid, primarily added in column and finally washed 6 times with 40 mL of distilled water in any case). The chemical analysis of each aliquot showed increased levels PO_4^{-3}, Cl^-, NO_3^-, SO_4^{-2}, F^- and Br^- in the 1stwash aliquot, followed by a significant decrease over washes. Zero concentrations were observed after 3 washes in almost all cases. Similarly, the increased levels of Zn, Be, Cs, Mn, V and Se determined in the 1st wash aliquot were eliminated followed successive washes. In parallel, the toxic potency of each wash aliquot was recorded by (a) a multi-well test plate bioassay, using instars II-III larvae of the fairy shrimp *Thamnocephalusplatyurus* (Thamnotoxkit F), (b) the Microtox bioassay, using bioluminescent bacteria *Vibrio fischeri*, andthe Cytokinesis Block MicroNucleus assay in human cultured lymphocytes. According to the results, 1stand 2ndbiochar aliquots/washes were toxic for *T. platyurus* (LC_{50} values of 22.12 and 68.28% v/v, respectively), followed by a significant elimination of toxicity after further washes in all cases. Similarly, the Microtox bioassay showed a significant inhibition of *Vibrio* luminescence after treatment for a period of 5-90 min (98-100% inhibition of luminescence) with the first wash aliquot (EC_{50} ≤ 0.01 % v/v), with no toxicity to be observed after successive washes. In addition, the CBMN assay showed that different concentrations(2.5-20 % v/v) of the 1stbiochar aliquot/wash were able to induce cytotoxic (in terms of CBPI index and the percentage of cytostasis) and genotoxic effects (in terms of the percentage of micronuclei formation in binucleated cells) in lymphocytes, followed by a significant attenuation over washes (2nd and 3rd washes, respectively), with values almost similar to those of control cells in all cases.The current findings revealed for the first time that biochars' sustainable use and application required a sophisticated elaboration/process for minimizing its potential toxic effects. Namely, at least two washes of biocharare prerequisite for improving its quality, thus being a crucial step for avoiding the induction of adverse effects on biota.

Microplastics, nanoplastics and co-contaminants: Fate, effects and risk assessment for biota, the environment and human health (P)

TH182

Microplastics' fate and potential effects in soil ecosystems: the need for future research

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Soils mediate various ecosystem services while being faced with the direct and indirect emission of a variety of pollutants and waste. Microplastics, small plastic particles < 5 mm, have attracted attention in the marine and freshwater environment but the terrestrial pathway has so far been forgotten. Thus, this study reviewed the scientific literature to provide a first insight on the fate and effects of MPs in soils. MPs enter soil by multiple sources, including aerial deposition, irrigation with contaminated water, fragmentation of plastic covers used in agricultural or horticultural products, and fertilization with sewage or sludge that contain MP particles from detergents and fibers from clothes. Soils may contain up to ~7 % (w/w) of MPs with polyethylene being the material most commonly found. The persistence of MPs (>100 years) is increased in soils due to low light and oxygen, e.g. MPs retained the original characteristics in soils fertilized with sludge 15 years after application. There is no published long-term monitoring study of MPs in soils, but data for marine coastal sediments suggest that MPs concentrations keep increasing worldwide. Only six studies investigated MPs in soils by the time of preparation of this abstract, with none of them addressing the soil microbiome. Thus, little attention has been given to MPs potential effects on the organisms accounting for most of the soil biodiversity and ecosystem services. The only study of MPs effects on a soil organism (*Lumbricus terrestris*) reported increased mortality and growth reduction (Lwanga et al. 2016). The mechanistic evidence available for toxicity of MPs in sediments and aquatic systems suggests that soil impacts can be expected. MPs could threaten soil ecosystems because they can be taken up by soil dwelling organisms and physically disrupt physiological functions. Due to the high sorption capacity of the plastic material MP might also play an important transport pollutant vector either for uptake into organisms or for exposing soil microbes to high contaminant concentrations. Literature that is so far available suggests that sources of MPs are likely to increase its concentrations in soils, which have the potential to impact soil chemistry and physiology of soil organisms. Since the risk of MPs in soils to soil biota and soil functions is still unclear research on fate and effects of MPs and associated pollutants in soils is urgently required.

TH183
Mass load of microplastics through freshwater in South Korea: sewage treatment plant (STPs) and Running water
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Plastic debris including microplastics in aquatic habitat is a growing global concern. Important one of recent relevant issues is the uncertainties in lifetime (fate and transport) and mass budget of plastics in the environment. The amount of microplastics observed in the ocean surface water is several orders of magnitude lower than estimated load to the ocean. This uncertainty can be improved by quantifying terrestrial load via running water and including the residence of microplastics in freshwater system. In this study, we investigated the distribution characteristics and abundance of microplastics in a running water and neighboring sewage treatment plant (STP) which discharge wastewater to the running water. We surveyed the surface water of the Han River at four stations from the upstream to the downstream using different sampling methods (i.e., manta-trawl net vs. pumping+filtration into phytoplankton net). Additionally, influent and effluent were investigated for four STPs. Abundance of MPs in influent and effluent of STP were 1000-10000 times and 10-100 times higher than surface water of running water, respectively. Only small portion (1-2%) of microplastics in influent was present in effluent. The most abundant microplastic changed from fiber in STP influent to fragment in STP effluent and surface water running water due to strong removal efficiency of fiber in treatment process and during water flows. This indicates the fractionation of microplastics to different environmental media. However, there is still problematic issue associated with sampling methods.

TH184
Sludge as an origin of microplastic in agricultural farmlands
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There is little known of microplastics in agricultural soils. This study focusses on occurrence of microplastic in the size range of 20-500 μm in soils which received wastewater sludge as fertilizer. It presents the method of sample preparation and identification of microplastics using FT-IR imaging techniques, but centers on data from a field study. The study investigates microplastic in 5 soils receiving sludge as fertilizer, 5 soils receiving traditional fertilizer and that which is found in sludge from 5 Danish wastewater treatment plants. In Denmark sludge can be applied to agricultural fields once every three years, and the amount of sludge received is determined by its' phosphate content. With the limit at 90 kg P/ha, the equivalent amount of sludge per hectare is approximately 10 ton. As recent studies have reported microplastics in wastewater, the need arises for determining the occurrence of microplastics in agricultural soils which have received sludge. The applied method in short first comprises microplastic extraction, where a sample is first suspended in a surfactant solution, agitated and wet-sieved into 2 size fractions of 10-80 and 80-500 μm. Each sample then goes through densimetric separation of the inorganic fraction using a 1.7 g/cm^3 zinc chloride solution. The organic fraction is subsequently oxidised using 30% H_2O_2 enhanced by a catalyst. The sample is filtered over a 10 μm mesh steel filter, and hereafter suspended in ethanol. Respective of their size fractions, samples are deposited on IR transmission windows or Kevley MirrIR reflection slides. After all ethanol is evaporated, samples are analysed using FT-IR imaging and subsequently identified by spectral interpretation. Preliminary data shows that sludge from the Danish wastewater treatment plants contain in the order of 5000 μg microplastic per gram sludge. Of this plastic found in sludge, approximately 80% was a type of polyethylene. Other common microplastics found were polyamides and polypropylene. The microplastics found in agricultural farmlands were of the same types, though the initial data show that the distribution of microplastic types was different. Whilst deep scanning of samples continues, the research should provide clarity on the relative contribution of microplastic from sludge to farmlands. The resulting data will be expected to show whether the distribution, shape, size and mass of microplastics in sludge are reflected in those fields that have received sludge.

TH185
Microplastics in terrestrial ecosystems: the impact of nylon microplastics in soil on earthworm reproduction
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Microplastics research to date has focussed mainly on the marine environment and only recently has more attention been paid to freshwater and terrestrial ecosystems. Terrestrial ecosystems can act as both sinks and sources of microplastics in the environment. Sources to the terrestrial environment include the application of sewage sludge to agricultural land, plastic mulching on fields and degradation of diffuse plastic litter. However there is still very little knowledge about the abundance of microplastics in terrestrial environments, how the presence of microplastics may impact terrestrial organisms and the ecological consequences of microplastics for soil invertebrates are poorly understood. The aim of this study was to investigate how microplastic particles in soil can affect earthworms, a keystone species in soil ecosystems, considering effects on their life history traits (survival and reproduction). The small earthworm *Enchytraeus crypticus* was exposed to a concentration range of nylon particles (20 – 120 g/kg soil) of two different size ranges (13-18 μm and 90-150 μm) for 21 days, after which earthworm survival and reproduction (based on number of juveniles) were measured. Earthworm survival was not impacted by either particle size range. Both particle sizes caused reproduction to be reduced in a dose-dependent manner, although an EC_{50} could only be calculated for the smaller particle (104 ± 8 g/kg). Ingestion studies showed that more of the smaller size range particles were ingested compared to the larger particles, which is likely to be related to the effects caused on reproduction. In a similar study *Enchytraeus crypticus* and *Eisenia fetida* were exposed to PVC particles in soil, but no effects on survival or reproduction were observed at the concentration 90 g/kg. Although the plastic concentrations used in these studies are high they are still within an environmentally realistic range, as plastic can, in some places, constitute up to 10% of the surface soil. In order to better understand how plastics are exerting effects it will be important to link these observed effects with the underlying mechanisms of toxicity.

TH186
Microplastics in a UK canal system: Abundance, characterisation and impact on freshwater microbial communities
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Microplastics (MPs), widely defined as plastic items < 5 mm in length (Thompson, 2015), are of global, environmental concern due to their persistence and prevalence in natural environments. To date, MPs have been detected in numerous marine habitats, such as Artic Sea ice (e.g. Obbard *et al.,* 2014), and deep-sea sediment (e.g. Van Cauwenberghe *et al.,* 2013), and a wide array of marine organisms, ranging from microscopic zooplankton (e.g. Cole *et al.,* 2013) to whales (e.g. Lusher *et al.,* 2015). Whilst MPs have been well documented in the marine environment, little is known about freshwaters despite the suggestion that rivers may be a primary source of MPs to the open ocean (e.g. Eerkes-Medrano *et al.,* 2015). \n There is thus an urgent need to address freshwater knowledge gaps by assessing the occurrence, fate and impacts of both MPs and nanoplastics (NPs) in selected freshwater systems. In this study, MPs were separated from sediment and surface water samples taken from the Worcester and Birmingham Canal, UK. MPs were then counted and subsequently categorised by polymer type, plastic type and size. Using the Biolog® multiwell microplate technology, selected and sorted (by size, colour, shape, density) MPs were exposed to freshwater microbial communities typically found in either sediment or water column habitats to ascertain whether the microorganisms could survive, metabolise and potentially degrade the MP polymers. Selected MPs were also exposed to chemical contaminants, e.g. plasticizers, insecticides, and then included in the assay to investigate any synergistic effects of plastic and co-contaminant. By analysing MP abundance in sediment and water samples, we can determine if sediment retains MPs, therefore limiting the amount of MPs that could flow downstream to other environments. Additionally, we can also determine the effect of MPs on sediment-specific or water column-specific freshwater microbial communities and how that could impact the fate of MPs in the environment.

TH187
Nanoplastics affect the toxicity of copper to the freshwater microalga Pseudokirchneriella subcapitata
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Plastic debris exposed to biotic and abiotic weathering processes undergoes

degradation and fragmentation into smaller particles, forming micro- (< 5 mm) and potentially nano- (< 100 nm) sized fragments. Due to their high surface–volume ratio, nano-scale plastic particles, might have greater capacity to adsorb organic compounds and trace metals than particles of bigger size, thus increasing the chance of uptake and toxicity of chemicals to exposed organisms. Nanoplastics also represent an emerging environmental issue because of their nano-size properties which give them specific toxic potential depending on surface characteristics and interactions with the surrounding medium. Carboxylated polystyrene nanoparticles (PS-COOH NPs) of a nominal size of 70 nm have been tested alone and in combination with copper nitrate to freshwater microalga *Pseudokirchneriella subcapitata* at different concentrations (5-25 µg Cu/L and 0.5-10 µg/mL PS-COOH) (acute toxicity test, OECD 202). Results show that the inhibition of growth exerted by copper is enhanced in the presence of PS-COOH NPs, even at those low nanoplastic concentrations that are not capable of inducing any effect when given alone. The growth inhibition curve also showed a clear dose-response relationship. DLS analysis has been applied in order to understand if and how the presence of microalgal EPS and/or Cu could change the behaviour of PS-COOH NPs in the freshwater medium. In order to examine the interaction of PS-COOH NPs with cell walls and the possibility of NP uptake inside the cells, SEM and TEM analysis were executed, with and without copper in solution. Furthermore, to investigate the capacity of the PS-COOH NPs to adsorb dissolved copper, ICP-MS analysis was carried out in presence and absence of NPs. Adsorption of copper by plastic and interactions of NPs with the cells are further analyzed.

TH188

Polyethylene microbeads evidently affect the Lemna minor root growth and root cell viability

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Plastic particles of different types and sizes have been detected worldwide. Smaller particles (< 5 mm), referred as microplastics (MPs), are one of the most discussed size class of plastic particles. Important amount of MPs can be found in wastewater effluents; they mostly contain polyethylene microbeads from used cosmetic products. Microbeads are released into the freshwater ecosystem and can affect different organisms. Polyethylene microbeads are usually floating on the water surface and are often accumulated in lakes and ponds. Therefore there is a potential to interact with floating plants. The aim of our study was to evaluate an impact of polyethylene microbeads extracted from a cosmetic product on floating macrophyte – duckweed *Lemna minor*. After seven days of exposure to polyethylene microbeads (10, 50, and 100 $mg \cdot L^{-1}$) no effect on specific growth rate of duckweed was found. Similarly, no significant reduction of photosynthetic pigment concentration (chlorophyll a and b) in comparison to control was observed. However, the length of duckweed roots significantly differed from the control at all concentrations. In addition, the viability of root cells was decreased as shown by Evans blue staining. The most plausible explanation for the observed effect is that roots of duckweed were not able to grow deeply in the presence of microbeads and were damaged by sharp shapes of the particles. The results of our study showed, that microbeads from cosmetic products can negatively affect the root growth of floating plants.

TH189

Fate and effects of microplastics in a freshwater mesocosm study

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The ubiquitous abundance of microplastics (MP) in freshwater ecosystems promotes the concerns for adverse impacts on aquatic biocenosis. So far, the recovered polymer materials from environmental samples illustrate a heterogeneous distribution of MPs throughout different compartments (surface, water column, sediment), whereby the sources, fate and fragmentation of MPs remains largely unknown. Additionally, our knowledge about biological effects is limited to simplified exposure regimes and, thus, hampers an evaluation of adverse effects in environmental relevant scenarios. Therefore, we conducted an outdoor mesocosm study with three different treatments. Next to a control, two additional treatments contained either virgin or conditioned MPs. The MPs (250-500 μm) were prepared by grinding and sieving green PET bottles, white PS drinking cups and blue LDPE pellets. To investigate the impact of conditioned MPs, we exposed 250-500 μm MPs to wastewater under laboratory conditions (48 h). After a two month acclimatisation period, the MP and conditioned MP treatments were spiked with a mixture of 150 particles L^{-1} PS, 10.7 particles L^{-1} PE and 10.7 particles L^{-1} PET. The environmental fate of MPs was evaluated by collecting and processing water and sediment samples. To reveal potential impacts on aquatic communities by MP exposures, zooplankton, macro-invertebrates and phytoplankton samples were collected in 2-week intervals over a period of 128 days. The conducted study aims for a thorough understanding of environmental implication by investigating multiple endpoints as well as the fate and fragmentation profiles of MPs in a freshwater model system.

TH190

Freshwater microplastics and competitive co-occurring pollutants

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Microplastic contamination of the marine environment has been widely acknowledged in recent years, and research has lately turned to investigate the impacts this type of pollution is having in freshwater ecosystems. Microplastics have been shown to have detrimental impacts on the health, functioning and survival of a range of marine and freshwater organisms (Avio *et al. 2015;* Besseling *et al.* 2014;Cole *et al.* 2013). In this study, we focused on the effect that microplastics can have on freshwater organisms including algae and daphnia magna (a keystone species) in the presence of other ubiquitous freshwater contaminants, such as 17α ethynylestradiol (oestrogen) and widely used domestic and industrial detergents. We compared the effects that different plastic densities and sizes (and therefore surface areas) have on the chemical transfer by quantifying the proteins and polysaccharides secreted by the organisms exposure to microplastics and 17α ethynylestradiol or detergent (singly and as mixtures); and (2) quantifying the uptake of the microplastics and 17α ethynylestradiol or detergent (singly and as mixtures). Under realistic exposure scenarios however, competitive absorption of chemicals onto nano and microplastics is another important facet due to the complex cocktail of chemicals currently found in the environment. We investigated the effect that competitive adsorption has on the uptake of 17α ethynylestradiol and detergents onto plastics in combination with other chemicals, including natural organic matter at different concentrations. The effect that biomolecules have on both adsorption and desorption of chemicals on the plastic's surface was also studied to ascertain an understanding of how chemically contaminated microplastics may be part of a more complex pollution issue in the environment. Together these studies are supporting the design of more realistic exposure scenarios under which to assess the toxicity and biological impacts of nano and microplastics and co-occurring pollutants on algae and Daphnia magna, to feed into regulation and freshwater management, including under the River basin management plans of the Water Framework Directive.

TH191

Combined toxicity of microplastics and nickel on Daphnia magna

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Plastic debris is of concern in aquatic ecosystem. They can be combined with other toxic pollutants, and they could be ingested by aquatic organisms as complex pollutants. In this study, we conducted combined toxicity tests using nickel and two types of microplastics. Test species is *Daphnia magna*. Test microplastics were microplastics with and without functional group. We used carboxylated microplastics (mPS-COOH). *Daphnia magna* were exposed to both nickel and microplastics for 48 hours, and adverse effects were observed. The results showed that mixture of nickel and carboxylated microplastics was more toxic that the mixture of nickel and microplastics without carboxylated functional groups to *Daphnia magna*. The reason could be the stronger combination of nickel and mPS-COOH by functional groups. Further study is needed to elucidate the mechanism of combined toxicity of microplastics and pollutants sorbed to the microplastics. *This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2016R1A2B3010445). This study was also funded by the Korea Ministry of Environment (MOE) as the Graduate School of Specialization for managing information of chemical risk.*

TH192

Single and combined effects of microplastics and chemical pollutants on freshwater biota

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Freshwater biota is facing pollution by chemicals, but also by particulate materials like microplastics (MP, polymer particles < 5 mm). In addition, MP contain chemicals (additives) that might leach out of the polymer material. Depending on the polymer type hydrophobic chemicals, e.g. used in households, also tend to sorb to MP. Together with MP like fibers from clothes or particles from cosmetic products they enter the environment via wastewater treatment plants. Filtering organisms and juvenile stages of freshwater biota seem to be especially vulnerable to stressors like water pollution. Thus, both their single effects as well as their resulting combined effects need to be studied to estimate the risks of MP and associated chemicals in the environment. Although experimental studies on the uptake of pollutants showed that MP can act as vector for pollutants, model based studies indicate that the vector function of MP is negligible in the environment. Laboratory studies under well defined conditions can help to systematically identify underlying processes by first studying single effects before combining them. In the first part of our project we analysed if and how effects on limnic zooplankton (*Daphnia magna*) of bisphenol A (BPA), an additive used in polycarbonates, are influenced by the presence of MP. In batch experiments with constant MP concentration and varying BPA concentrations (5-20 mg L-1) the concentration of BPA in water decreased until sorption equilibrium was reached. The effect rate of the daphnids (immobilisation) followed the concentration gradient of BPA in water, although daphnids ingested MP loaded with BPA. These results indicate, that the vector function of MP does not induce effects within the aquatic organism. In contrary, the combination of BPA with MP even causes lower effects than BPA alone. In the next part we analysed single and mixture effects of 17α-ethinylestradiol (EE2), an endocrine disruptor used in contraceptives, and MP on the amphibian *Xenopus laevis* as one representative of freshwater vertebrates. We focused on effects on development and sex determination regarding both morphological and biomarker analysis. First results from batch experiments show that exposure of MP alone does not seem to influence morphological parameters. The results of this study show how MP and pollutants mutually influence their effects on limnic organisms and thus provide insights for an indepth risk assessment of MP in the environment.

TH193

Tissue transfer potential of Microplastics in Daphnia magna

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Over the past decade the pollution of aquatic environments with microplastics (MPs, particles < 5 mm) has emerged as a major concern in ecology and ecotoxicology. Most of the research so far focused on the uptake of macroplastics by megafauna and only recently the focus partly shifted towards lower trophic levels. At the same time, the majority of research is directed at the marine environment. Multiple studies have found MPs in lake and river sediments across North and South America, Europe and Asia. Within the frame of the MiWa project, funded by the German Federal Ministry of Education and Research, we investigate the impact of microplastics on freshwater invertebrates. As a first step, we assessed the hypothesis that very small microplastic particles < 10 μm will transfer from the gut to adjacent tissue of freshwater model organisms such as the crustacean *Daphnia magna*. The majority of uptake studies use ready-made spherical microbeads, which are not representative of particles shaped through environmental impact. Our approach was to use irregular plastic fragments, produced by cryo-milling and sieving to achieve a more realistic exposure scenario. Exposure occurred short-term (< 24 h) and longer-term (> 96 h) and animals were then investigated using confocal laserscan microscopy to attain higher resolution and depth of field than with classic fluorescence microscopy.

TH194

Size matter? Physical effect of large microplastic particles (Polystyrene) on Daphnia magna

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Microplastics are present in many commercial products such as toothpaste, facial cleanser and body scrubs.Plastics transferred from wastewater treatment plants to surface water ending up polluting both marine and freshwater habitats. Several recent investigations have shown that these particles vary in size and shape (Napper et al., 2015). My previous results has demonstrated the uptake and excretion of 2 μm polystyrene particles, these where non-toxic to adults *Daphnia*. Here I assesses the uptake and excretion of 15μm microplastic (polystyrene) beads on the model freshwater aquatic crustacean *Daphnia magna*. Epifluorescent microscopy and direct counts of beads show accumulation of microplastic particles in the gastrointestinal tract. This accumulation is linked to blockage of gut system and significantly mortality to *Daphnia* over time.

TH195

Microplastic: threat for freshwater Amphipods?

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Microplastic particles receive increasing attention in aquatic research regarding their potential toxicity. We studied the uptake and toxicity of a series of different polymers (different sizes, structure, color) by *Gammarus fossarum* in the laboratory. Three types of plastic materials (PBT, PP, PPO/PS) were taken up the most, thus they were further investigated regarding their toxic potential (survival, behavior, feeding) during 17 d of exposure in the laboratory. We compared particles with and without ageing (biofilm formation) and particles with spiked TBT. Particles below 1 mm were preferred by *G. fossarum*, however, generally only few particles were taken up in spite of ad libitum dosage. Feeding on leaves was not affected. No difference between fresh and aged particles was found regarding uptake and toxicity. For PBT and PPO/PS the TBT-spiked plastic materials were more toxic than the particles themselves. In order to get an idea about the relevance of microplastic in the field, *D. villosus* from the shore of Lake Constance were investigated regarding their plastic contents in the gut by visual microscopic analysis: only in 2 out of 37 animals single fibres were found, in contrary to the mussel *Dreissena polymorpha*, taken from the same site, where each of the 50 studied animals contained several particles. We recommend further field studies under realistic conditions to better evaluate the risk of microplastic for different aquatic invertebrate species.

TH196

Release of microparticles from artificial turf into storm water and the biological effects of these particles in two biological systems: gut function of rainbow trout and LC50 toxicity in D. magna

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The presence, occurrence and fate of microparticles in the environment is a subject of much research. Several sources of microparticles have been suggested, including spread of particles from playing fields using artificial turf. This turf consists often of both polyethylene as well as rubber. We have conducted a study quantifying the number of particles released from three different playing fields in the city of Gothenburg, Sweden, by sampling runoff water in storm water drains, characterizing these particles, and then subsequently, using the rubber granulate in laboratory exposure studies with rainbow trout (*Oncorhynchus mykiss*) and water flees (*Daphnia magna*). The particles we studies consisted of EPDM rubber, also known to contain carbon black (31%) and ethylene propylene copolymer (26%). Fish were fed a diet consisting of commercial fish pellets containing 5% rubber granulate for a period of 1 week. Their guts were excised and function was analysed ex vivo using Üssing chambers. D. magna were used to determine LC50 acute toxicity, according to OECD Daphnia toxicity test 202, of water extractions of the particles. Our results showed that >100 particles (+/K^+-ATPase activity were noted, no changes in plasma ion concentration were found. No changes were seen in reduced or oxidized glutathione levels in the gut tissue. These results indicate that artificial turf may in fact be a significant source of microparticles in the environment, and that these have the potential to negatively impact biota.

TH197

Effects of polystyrene microplastics in brown trout (Salmo trutta f. fario) and the giant ramshorn snail (Marisa cornuarietis)

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The widespread use of synthetic polymers in our daily life has led to a constant increase in the production of plastic. The high amount of produced plastic and its durability has led to a ubiquitous occurrence of plastic debris in the environment. Abrasion and fragmentation of larger plastic items leads to the formation of microplastic (plastic items smaller than 5 millimeters). Consequently, microplastic as like larger plastic items nowadays occurs in the environment worldwide. Most studies investigating the effects of microplastic in organisms concentrate on the marine habitat whereas there is a particular lack of knowledge of the effects of microplastic in freshwater organisms. The present study is part of the joint research project "MiWa" (microplastic in freshwater systems) funded by the German Federal Ministry of Education and Research (support code: 02WRS1378). The aim of our study is to investigate effects of polystyrene microplastics and the toxicity of the combination of organic pollutants and polystyrene microplastics in the giant ramshorn snail (*Marisa cornuarietis*) and in brown trout (*Salmo trutta* f. *fario*). We examine the embryo toxicity of polystyrene microplastic particles in *Marisa cornuarietis* with the MariETT assay, and in sac-fry stages of brown trout (*Salmo trutta* f. *fario)* by means of the FELST. Investigated endpoints are the formation of eyes and tentacles (the latter only for *Marisa cornuarietis),* heart rate, hatching success and mortality. Furthermore, we examine the influence of microplastic on the level of oxidative stress in both species. Moreover, possible additive, synergistic, or antagonistic effects of the combination of polystyrene microplastic and a pesticide are investigated in adult individuals of *Marisa cornuarietis*. Here, effects on the mortality rate and biometric parameters are examined. Furthermore, biomarkers for oxidative stress and the 70 kD stress protein (Hsp70) level are determined. Additionally, the inhibition of acetylcholinesterase as a biomarker focusing directly on the pesticide´s mode of action is monitored.

TH198

Microplastic ingestion by brown trout living in a river polluted by industrial plastic production

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As plastic production increases worldwide, reaching over 311 million tons in 2015 (PlasticsEurope), so does plastic pollution. The occurrence of microplastics in the marine environment has been the subject of intense study in recent years, and these particles are found in a wide range of environmental compartments.The purpose of this study was to address release of primary plastics from an industrial production site, and to determine whether microplastics are ingested by the population of brown trout, *Salmo trutta,* living in the area. In this study we investigated two different rivers, the first receiving industrial waste water from factory site, and the second in area which is not directly connected to the factory, but in a region where storage, loading and transport of pellets and plastic materials occurs. We have documented pellet pollution photographically in the external perimeters and outside these companies, including around stormwater drains. Brown trout were collected at 4 sites using electrofishing, fish were killed and gastrointestinal tracts were removed. We treated samples using a method based on enzymatic digestion followed by treatment with hydrogen peroxide to remove all organic material. Samples were then filtered and analysed microscopically for microplastics. Representative particles were analysed using FT-IR to identify polymers. Polystyrene (PS), poly(methyl methacrylate) (PMMA or acrylic) and polyethylene (PE) particles were identified. We found evidence of a higher rate of microplastic ingestion in the fish dwelling downstream from the industrial site, pointing to the plastics production site as a likely source of pollution. Industrial release of microplastics is primary source of plastic pollution in the environment, and one that is relatively easily curbed through policy changes, awareness and behavioural changes among handlers. We emphasize the importance of addressing questions concerning microplastic pollution on multiple levels and are currently, together with colleagues, addressing several of these, including fate of microplastics in the environment, uptake in animals, biological effects of exposure, as well as addressing weaknesses in current regulations and policy and educating the general public.

TH199

Effects of ingested microplastics with sorbed triclosan on fish gut health and assessment of triclosan bioavailability

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Microplastics (MPs) are plastic particles (5 mm –1 μm) that are present in aquatic environments and can have negative effects in organisms. Potential effects of MPs include disruption of gut physiology after ingestion, release of substances (co-contaminants) sorbed to MPs into organisms, and occlusion of tissue surfaces by accumulation of MPs. The effects of MPs after ingestion are not well understood and there is no information on whether MPs can affect gut microbiota or if this disruption can influence immune system responses. Triclosan (TCS) is a widely used antimicrobial hydrophobic compound that has been detected sorbed to polyethylene aquatic debris. Exposure of fish to TCS in aqueous phase (even at low doses, < 100 ng/L) can disrupt fish gut microbiota. The goal of this study was to assess the bioavailability of TCS sorbed to high-density polyethylene (HDPE, 125 - 250 μm) MPs after 0 - 5 days of ingestion in rainbow trout *Oncorhynchus mykiss*. After 7 and 14 days, the effects of MP ingestion on gut microbiota, intestinal epithelium integrity and immune system response were evaluated. The TCS sorption to HDPE was assessed by measuring TCS concentration changes in aqueous phase using high-performance liquid chromatography (HPLC). The detected sorption after 24 h was of around 20% to PVC particles (TCS initial concentration of 0.1 mg/ml). Measurements of target gene expression by quantitative reverse transcriptase PCR (RT qPCR) were used to assess TCS bioavailability and immune system response. The bioavailability of sorbed TCS was assessed by measurement of glutathione S-transferase (GST) expression in liver of fish, and the immune system response was assessed by measurement of the expression of serum amyloid A (SAA) in the liver and Immunoglobulin M (IgM) in the spleen. Intestinal epithelium integrity was examined by histopathology. Finally, alterations in the gut microbiota were assessed using Terminal Restriction Fragment Length Polymorphism (t-RFLP), a sequencing based technique for rapid profiling of microbial communities, after DNA extraction and amplification of 16S rRNA gene fragments using PCR. We anticipate that our results (ongoing data analysis) will contribute to a better understanding of the fate and effects of MPs and their co-contaminants in aquatic environments.

TH200

Interactions between microplastics and biota in the marine environment

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Microscopic plastic (microplastic) debris is a widespread marine pollutant that poses a threat to aquatic biota and healthy ecosystems. Microplastics have been detected throughout the world’s oceans; however, the relative importance of different processes in controlling the spatial distribution and long term fate of microplastics in the marine environment remains largely unknown. We hypothesise that biological interactions are key to understanding the movement, impact and fate of plastic in the oceans. We provide an overview of the different mechanisms through which marine life and microplastics can interact. Laboratory and field studies demonstrate microplastics can have significant impacts upon biota, but we highlight that such interactions may also play an important role in plastic redistribution through consumption, egestion and aggregation of plastics. Using global microplastic sampling data and sea surface chlorophyll as a proxy for biological productivity, we highlight biomes where interactions are most likely occur. We predict that the overlap between buoyant microplastics and biota will be most prevalent in shelf sea regions, owing to the biological productivity of coastal waters and their proximity to sources of anthropogenic pollution [1]. Developing a better understanding of the interactions between biota and microplastics is an urgent research goal for improving the management and protection of our oceans from the emerging hazards posed by marine litter. [1] Clark et al. Front. Ecol. Environ. 2016, 14, 317–324.

TH201

Ecological investigations of microplastics and microparticles in environmental matrices - the role of particle properties

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Investigations of the environmental fate and effects of microplastics and microparticles is now in vogue. Microplastics are defined as small, insoluble, synthetic polymers with size

TH202

Uptake and effects of microplastic particles in selected marine microalgae

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Microplastics (MPs) are commonly defined as microscopic plastic particles sized between 100 nm and 5 mm; a size range covering many plankton species including microalgae. Interactions between MP particles, algal cells and their planktonic predators can therefore not be excluded. Microalgae are keystone organisms in marine ecosystems on which the whole food chain depends. Consequently, impact assessment of MP pollution on the marine environment warrants the study of effects on lower trophic levels of marine ecosystems. Populations of selected microalgae species including the cryptophyte *Rhodomonas baltica* and the dinoflagellate *Oxyrrhis marina* are therefore exposed to environmentally relevant concentrations of comprehensively characterized 10 um particles of low density polyethylene (LDPE) and general purpose polystyrene (GPPS). Custom-made transparent plankton wheels immersed in a water bath are used to expose these phytoplankton species in a (semi-)static setup in order to keep MP and algal cells in suspension, and maintain constant temperature and light conditions. Exposure effects on biomarkers of fitness with ecological consequence are studied using *R. baltica*. For example effects on growth/production are evaluated by measuring changes in growth rate and population size (cell number), biomass (cell size), maximum carrying capacity (maximum number of algal cells in the population), pH change in the exposure media, and chlorophyll production. In addition, MP uptake by *O. marina* is investigated and its effect on *O. marina* sinking speed measured.

TH203

Chronic ingestion of environmentally relevant doses of microbeads has no significant effect on growth to intertidal amphipod Echinogammarus marinus

S. Bruck, University of Portsmouth / School of Biological Sciences; A. Ford, University of Portsmouth / Biological Sciences

Plastic pollution is considered one of the major current anthropogenic threats to marine life. Microscopic plastic particles (microplastics) that have fragmented from larger plastics or been produced miniscule in size, are of particular concern as they may be bioavailable even to organisms of planktonic size. The ingestion of microplastics has been observed in a range of marine organisms, and adverse effects of high concentration exposure have been reported in several species. However, the chronic effects of low-dose ingestion remains unclear. This aim of this study was thus to assess the chronic effects of environmentally relevant concentrations of microplastics to the intertidal amphipod *Echinogammarus marinus*. Fifteen amphipods per treatment were fed a gelatinous algal feed spiked with fluorescent microbeads (8μm) in concentrations of 1, 10 and 100 particles/ml for 35 days in 3 replicate tanks. It was hypothesised that the contaminant would interfere with digestive functions and thus alter feeding rate and metabolism, and consequently disrupt the moulting cycle and reduce body weight. Food consumption, growth, and moulting was therefore used as endpoints, and were measured at regular intervals throughout the experiment. Retention of microbeads was assessed upon completion of the trial period. Microplastic egestion rate was also calculated in a separate high-dose feeding experiment. No significant effects (ANOVA $p > 0.05$) were found in the food consumption or growth assays. In 8% of the animals, only one single microbead was found in the gut after 35 days of exposure. The low number is likely linked to *E. marinus'* gastrointestinal functions, allowing for easy egestion of indigestible items. This assumption was supported by the observation that 70% of the amphipods egested all ingested microbeads in less than 24 hours. A significant difference was found in the cumulative timing of moulting events in the low and the high exposure groups as compared to control (K-S Test $p < 0.05$). As the finding was not supported by the other data, the repeatability of this experiment would need to be confirmed. Overall, results indicate that within the parameters set for this experiment, that 8μm microbeads are readily egested and do not represent an immediate environmental risk to growth or feeding in *E. marinus.*

TH204

Uptake and effects of microplastic particles in the marine copepod Calanus finmarchicus

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The marine copepod *Calanus finmarchicus* is a filter feeder and a keystone species of the Northern Atlantic. Microplastics (MPs) are commonly defined as microscopic plastic particles sized between 100 nm and 5 mm, a size range covering the preferred prey size of *C. finmarchicus*. Hence, interactions between MPs, microalgal cells and their zooplanktonic predators cannot be excluded. *C. finmarchicus* are therefore exposed to environmentally relevant concentrations of comprehensively characterized 10 mm pellets of low density polyethylene (LDPE) and general purpose polystyrene (GPPS) in both the absence and presence of microalgae. Custom made transparent plankton wheels immersed in a water bath are used to expose this calanoid in a (semi-)static setup in order to keep microplastic and algal particles in suspension and maintain temperature and light conditions constant. Ingestion, excretion and accumulation rates of MPs in *C. finmarchicus* are quantified. In addition, exposure effects on biomarkers of fitness with ecological consequence are studied. For example effects on naupliar growth and development rates are investigated as well as effects on adult female fertility and fecundity.

TH205

Do recycled rubber tires pose a risk for the marine environment? A chemical, physical and effects assessment

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Globally, car tyres have been identified as one of the most significant sources of microplastic (MPs) particles to the environment. Due to the large production volumes and their persistent nature, the disposal of used tyres has also been a major challenge in waste management, making tyre recycling a necessary alternative to landfill disposal. In Norway alone, approx. 55 000 tons of tyres were collected for recycling in 2015. Current uses of recycled tyres include conversion to rubber mulch or granulated particles (< 5 mm) prior to use in asphalt production for road construction, flooring in playgrounds and application on artificial grass sports fields. The latter uses have seen increased volume in recent years due high profit gains on recycled materials generated from the abundant and low cost waste tyres. However, tyre rubber contains a complex mixture of chemicals including highly toxic compounds such as aromatic oils, reactive additives, metals, and PAHs. In addition to generation of MPs during normal use, anthropogenic processing of waste tyres represents another significant source. This causes increasing concerns, not only regarding potential risks for human health but also for environmental exposure. In this study, we use pyrolysis GC-MS, GC-MS, ultrahigh resolution FT-ICR-MS and triple quadrupole ICP-MS-MS to investigate the concentrations of potentially toxic compounds (additives and PAHs) and elements in new and used granulated tyres. The leaching of potentially hazardous compounds from three commercially available granulate sizes will be investigated under conditions relevant for the marine environment, i.e. seawater, sediment and the seawater-sediment interface. Impacts of physical stress on MP degradation and leaching of chemicals will be investigated. The uptake and potential adverse effects of both, fresh and naturally weathered fine-grained MPs from tyres will be studied on *Hediste diversicolor*,a marine sediment dwelling species and potential primary target organism. Furthermore, toxic effects of the leachates will be investigated on the pelagic crustacean *Calanus finmarchicus*, a key species in the North-Atlantic food web.

TH206

Do microplastic fibers exert a greater toxicity than microbeads?

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Microplastics are ubiquitous in the marine environment and have accumulated in ocean habitats worldwide. Owing to their small size microplastics can be ingested by a wide range of marine organisms from zooplankton to seabirds. Although over 267 marine species have been recorded to ingest microplastics the biological effects remain largely unknown. Many microplastic shapes, types and sizes are reported in the marine environment, and despite fibrous shapes being the most frequently reported (2-31 fibers /250ml sediment), the majority of laboratory based exposure studies are conducted using spherical beads at high concentrations. This study investigated the differences in biological effects between ingested microplastic beads and fibers in the ecologically important polychaete worm *Hediste diversicolor*. Sediments are hypothesized to be a major sink of microplastics and therefore have the potential to interact with benthic dwelling organisms. *H. diversicolor* were exposed to spiked sediments containing microplastic beads and fibers (polyamide/polypropylene/polyvinylchloride) at a size range of 50 – 3000µm for 48 hr and uptake was measured. A range of biological effect endpoints were also measured including those indicating inflammatory responses. Here we present and discuss the differences in uptake and biological responses between microplastic fibers and beads across differing size ranges and its consequences in the marine environment. The impact of microplastic ingestion on biota occupying low trophic levels, such as marine polychaetes, has until now remained largely unknown despite their significance in marine food webs. Hence the ecological importance of assessing microplastic ingestion and its impacts on these key species.

TH207

Accumulation and biomarker responses of mussels Mytilus galloprovincialis to polystyrene microplastics and adsorbed organic and metallic contaminants

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Marine filter-feeding organisms, such as the mussel *Mytilus galloprovincialis*, are considered target species for microplastic (MP) pollution. Ingestion of MPs could lead to their accumulation into the digestive system, disturbing feeding processes, and ultimately to their internalization into the tissues. Moreover, MPs could act as vectors for pathogens and other pollutants present in the water column and sediment. Since mussels are an appreciated food resource, the assessment of MP pollution is an issue of human health concern. This work aimed to provide preliminary data on (1) the influence of MP size (4.5 vs 45 μm Ø) and concentration (1, 100 and 1000 particle/mL) on their accumulation and on the mussel depuration ability, and (2) the role of MPs as carriers of organic (benzo(a)pyrene, BAP) and metallic (Cd) pollutants. The histological assessment showed that after 1-day exposure MPs were accumulated in the digestive gland in an exposure concentration-dependent manner. The larger particles were found in the stomach and primary ducts, whereas smaller MPs were found in the connective tissue surrounding the digestive tubules and gonads. Some MPs were observed between the gill filaments. After 3-day depuration, some MPs were still present in the tissues. Mussels exposed for 3 days to MPs of 4.5 μm previously incubated with BAP (1 μM) showed higher BAP levels than control mussels, whereas Cd accumulation was not detected in mussels exposed to MPs previously incubated with Cd (1 μM). This indicated higher capacity of polystyrene to adsorb BAP than Cd. Plastics were also mainly localized in the connective tissues and along the gill filaments. Short-term exposure to polystyrene MPs alone or in combination with BAP or Cd did not provoke significant alterations in the activities of the antioxidant enzymes catalase and superoxide dismutase neither in the digestive gland nor in the gill and no relevant histopathological alterations were observed. In conclusion, these short-term studies provided evidence that MPs are differentially uptaken and internalized by mussels depending on their size and that MPs act as carriers for organic persistent pollutants, such as BAP. These results encourage us to perform longer-term studies to investigate cellular and physiological effects of this emerging type of pollution. Study carried out in the framework of JPI PLASTOX project, funded by Spanish Government (CTM2016-81130-R), Basque Government (IT810-13) and UPV/EHU (UFI 11/37 and VR for Research).

TH208

Uptake and toxicity of polystyrene nanoplastics compared to microplastics, alone and with adsorbed benzo(a)pyrene, in hemocytes of mussels Mytilus galloprovincialis

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Plastics are synthetic polymers widely used in our daily lives that cause serious global pollution problems due to their accumulation in the environment, specifically, in the oceans. Mechanical, chemical and biological degradation of large plastic items causes the formation of plastic particles smaller than 5 mm, known as microplastics (MPls). In addition, MPl particles are manufactured for specific purposes, such as personal care products, contributing to the appearance of plastic debris in the ocean. These MPls can be ingested by invertebrates and cause damage to them. Moreover, occurrence of nanoplastics (NPls, < 100 nm) in the marine environment is expected to grow in the next years as a consequence of degradation of larger particles or due to the development of nanotechnology industry. It has been suggested that NPls and MPls could act as carriers of persistent organic pollutants (POPs) in the ocean causing the so-called Trojan-horse effect, because POPs could be adsorbed and concentrated in plastics' surface. The aim of this work was to compare the uptake and toxicity of polystyrene NPls (50 nm) and MPls (0,5 and 4,5 mm), alone and with adsorbed benzo(a)pyrene (bap), using primary cultures of hemocytes, immune cells of mussels *Mytilus galloprovincialis*. Uptake of MPls by cells was confirmed by inverted and polarized microscopy. Exposure to a wide range of NPl and MPl concentrations (100 to 10^6 particles/ml)

did not produce a decrease in cell viability but an increase in cell viability (at 24 h) and ROS production (already at 1 h, for 50 nm NPls). Exposure to NPls and MPls with adsorbed bap (1 and 158 mM) caused a significant decrease in cell viability and increase in ROS production at the highest bap concentration, especially in the case of 0,5 mm MPls. Overall, results indicate the potential of polystyrene NPls and MPls to act as carriers of bap and to cause deleterious effects on mussel immune cells. Study carried out in the framework of JPI-Ocean PLASTOX project, funded by Spanish Government (project CTM2016-81130-R), Basque Government (consolidated research group IT810-13) and UPV/EHU (UFI 11/37 and VRI special action).

TH209

Development of a dynamic uptake model for virgin and contaminated microplastics in the Mediterranean mussel (Mytilus galloprovincialis)

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Over the last few years, microplastic pollution of the marine environment has been highlighted as a major issue. Microplastic particles have been detected in a large number of aquatic habitats worldwide. Due to their small size, microplastics are often mistaken for food, and ingested by biota; their porosity and lipophilicity grant them the ability to soak hydrophobic persistent pollutants from the surrounding water, delivering these hazardous contaminants to the organisms consuming them. Very little is known concerning the mechanisms ruling microplastic ingestion and accumulation in biota: this important gap constitutes the missing link between microplastic concentration in the environment and organismal effects and toxicity in achieving a valid risk assessment, both for biota and for human health. Thus, a first dynamic model of microplastic uptake and accumulation was developed in the Mediterranean mussel (*Mytilus galloprovincialis*), a species relevant both on an ecological and an economical level. Mussels were exposed to polyethylene (PE) microplastics, of a size (3-12 μm, mean particle size 7.5) comparable to that of microalgae which constitute the natural diet of these organisms. A control of mussels exposed to microalgae (*Isochrysis* aff. *galbana* clone T-ISO) was also present. During the experiment, a microalgae and particle concentration below the pseudofaeces threshold reported for this animal was used, in order to encourage particle ingestion and avoid saturation. Furthermore, we selected the insecticide chlorpyrifos as a model contaminant, and exposed mussels to PE microplastics spiked with this compound, to understand if the presence of adsorbed persistent pollutants affects uptake and accumulation of microplastics. The preliminary results obtained in this study confirmed ingestion of microplastics by treated mussels, and highlighted a difference between microalgae and microplastics uptake and excretion rates. Overall, the dynamic model allows to simulate and predict the impact of microplastic exposure on uptake, retention and elimination rates under different exposure scenarios. For the future, there subsists a need for more dynamic models, encompassing different microplastic types and shapes (such as fibres) and organisms, to contribute to a better understanding of the threat posed by microplastic pollution, and to produce more accurate risk assessments.

TH210

Occurrence, amount and characterization of microplastics in the gastrointestinal tract of wild stocks of the common sole Solea solea in the Adriatic Sea (Mediterranean)

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Keywords: Microplastics, stomach content, common sole, Adriatic Sea. **Abstract** Large amounts of small fragments of plastic bags, bottles and clothes in the world's oceans represent a serious threat to human health and marine ecosystems. Once released into the aquatic environment, plastic litter is broken down to smaller pieces through mechanical and physical action of UV light and waves. The resulting particles may become so small that they are readily taken up in the digestive system of fish, crustaceans, or mussels in the aquatic environment. There is mounting evidence of the occurrence of plastic particles in marine organisms that are part of the human food chain, and this might also represent a potential threat to human health via biomagnification. In this work, we aimed at investigating the occurrence, amount, characterization and spatial distribution of microplastics in the gastrointestinal tract of natural stocks of common sole *Solea solea* collected in Adriatic Sea (Mediterranean). Plastic particles were scored and divided according to three dimensional classes: >100μm, 100 μm < X < 500 μm and < 500 μm. The digestive tract contents of 531 individuals collected from 60 sites between 2014 and 2015, both in coastal and marine areas, were examined. Plastic particles were found in almost all samples, and approximately 85% of fish had ingested more than one microplastic item. The most commonly found polymers' fragments were polypropylene and polyvinylchloride, followed by polyethylene and polyamide while polyethylene was the most recurrent polymer in fibers. A peculiar spatial distribution was observed while assessing the abundance and the relative composition. Preliminary results point out two potential hot spots within the observed spatial distribution.

TH211

Histological evidences for mechanical and physical damages induced by microplastics in the intestine of European sea bass, Dicentrarchus labrax (Linnaeus, 1758)

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Several studies have investigated the possible link between microplastics effects and the risk for the health of marine organisms. Despite some research have been performed to evaluate the physical impact of microplastics ingestion in marine organisms, available information about fish is still very poor. An experiment was conducted at IAMC-Messina aquaculture plant to investigate the physical effects induced by the ingestion of microplastics in the intestinal tissue of European sea bass, *Dicentrarchus labrax*. A total of 162 *D. labrax* (140±8.42 g) were randomly subdivided into 9 indoor tanks (3 replicate tanks for treatment). The fish were fed for 90 days with 3 different dietary treatments administered at 1.4% of body weight: food pellet without plastic as control (CTRL); food pellet supplemented with 0.1% (w/w) of native polyvinyl chloride PVC (MPV); food pellet supplemented with 0.1% (w/w) of PVC incubated for three months in the Milazzo harbour to obtain polluted PVC (MPI). The food pellets were manufactured in the laboratory using a basic diet for *D. labrax*; PVC samples were added to the experimental food pellets after being grinded (< 0.3 mm). The effects of the exposure were assessed by a histological and histomorphometric analysis. The functional integrity of the intestinal mucosa was evaluated by using, as morphometric index, the number of goblet cells. Cells have been counted in a tissue area of 600.000 μm^2 in 2 fishes for each treatment and replica at 3 sampling time (30, 60, 90 days) for a total of 54 samples. Chronic exposure to PVC caused pathological alterations in all samples, especially in the distal part of intestine (DI) and the numbers of goblet cells in DI increased in both experimental treatments compared to the control group. The results of PERMANOVA (one-way) showed significant differences ($p< 0.05$) between the control group and the experimental treatments at all the exposure times, while no differences were found between the two experimental groups. These first finding demonstrates that the intestinal functions can be reduced and, in some cases compromised. Moreover the increase of goblet cells in both experimental groups could be represents a defence strategy against mechanical and physical damage caused by microplastics. Further studies are needed to understand the consequences of this impact on marine ecosystems and also to evaluate additional adverse effects of microplastics ingestion on fish, in particular on edible species.\n \n \n

TH212

Impact of plastic debris on whale sharks (Rhincodon typus) from the Gulf of California (Mexico): ecotoxicological investigation using skin biopsies

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The impacts of plastic pollution (including microplastics) on large filter feeder marine species are largely unknown. The whale shark (WS) (*Rhincodon typus*) is an endangered (IUCN) species that may be exposed to micro- and macro-plastic ingestion as a result of their massive filter-feeding activity, particularly on the sea surface. The increasing human activity in WS grounds gives rise to chemical pollution and waste including plastic debris. Here we perform the first ecotoxicological investigation on WS sampled in the Gulf of Californiaexploring the potential interaction of this species with plastic debris (macro-, micro-plastics and related sorbed contaminants). Due to the difficulty in obtaining stranded specimens an indirect approach, based on the assessment of chemical tracers of plastic impact (eg. PBDEs) and related biomarkers responses (such as CYP1A) in skin biopsies, was used. Twelve WS skin biopsy samples were collected in January 2014 in La Paz Bay (BCS, Mexico) and a preliminary investigation on microplastic density and polymer composition was also carried out in the same areas.PCBs (twenty-one *ortho* PCB congeners), DDTs, PBDEs (fourteen congeners from tri- to deca-substituted) and HCB were analysed by GC-qMS. Cytochrome P450 1A (CYP1A1) was analyzed in skin biopsies, using western-blotting (WB) technique. The average abundance pattern for the target contaminants was PCBs>DDTs>PBDEs>HCB. Mean concentration values of 8.42 ng/g w.w. were found for PCBs, 1.31 ng/g w.w. for DDTs, 0.29 ng/g w.w. for PBDEs and 0.19 ng/g w.w for HCB. CYP1A1 was detected, for the first time, in WS skin samples. First data on the average density of microplastics in the superficial zooplankton/microplastic samples showed values ranging from 0.00 items/m^3 to

0.14 items/m^3; furthermore, concentrations of mono-(2-ethylhexyl) phthalate, used as a tracer of plastic additives, ranged from 13.08 ng/g to 13.69 ng/g. Theoretical number of microplastics assumed daily for WS is one/two order of magnitude less than Mediterranean large filter feeders such as basking sharks. The hypothesis of the ingestion of macroplastics as a main cause of plastic debris impact during the continuous surface feeding activities needs also to be explored in the study areas. Further ecotoxicological investigation on WS skin biopsies will be carried out for a worldwide ecotoxicological risk assessment of this endangerd species including plastic additive and gene expression biomarker detection.

TH213

Styrene oligomers (SOs) in polystyrene based consumer products: Tracing major contributor to environmental SOs

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Plastic debris is global emerging issue. The results of recent studies suggest a large uncertainty in global mass budget of plastics. This can be partly due to uncertainties in the plastic measurements in environmental media that depending on extraction and counting of individual plastic particle. Alternatively, chemical markers can use as a surrogate to trace plastic pollution. Styrene oligomers (SOs) including styrene monomer (SM), styrene dimers (SDs), and styrene trimers (STs) are not only the basic skeleton that constitutes polystyrene (PS), one of major plastic polymers produced globally, but also added as an impurity. The SOs can flow into the environment as PS progressively breaks down by weathering. Recent studies have showed the presence of terrestrial input of SOs, in addition to the possibility of leaching from plastic litters deposited on the beach. Variability in sources of SOs influence the distribution and composition profile of SOs determined from environmental samples. In this study, we collected a variety of consumer products including expanded PS (EPS), extruded PS foam (XPS), and extruded PS and determined the contents of SOs in each consumer product. For that, we applied the sedimentation technique, which consists of dissolution in DCM and sedimentation by hexane. The levels and composition profile of SOs determined in our study will be compared with those in environmental samples to trace the major pollution contributor. In the next step, we will proceed with leaching experiment from each consumer products.

TH214

Quantitative analysis of phthalate esters in plastic marine debris and fresh plastics using HPLC-TOF/MS

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Phthalate esters (PAEs) are widely applied to various plastic products as plasticizer and softener. PAEs are not chemically bound to the polymeric matrix but phyically mixed; consequently, they can leach, migrate or evaporate into the surrouning environment during their use or after disposal. PAEs have become an environmental concern due to their ubiquitous presence and their potnetial toxicity. Plastic debris has become a growing problem in the marine environment. One of key issues is whether plastic marine debris and its small fragments are a source or vector of hazardous chemicals to marine environment and marine organisms. However, there is limited information available on plastic associated chemicals, particularly additive chemicals. Based on previous non-target screening analysis of marine plastic debris, three phthalate esters [di(2-ethylhexyl) phthalate (DEHP), diisobutyl phthalate (DNOP), di-n-octyl phthalate (DBP)] were selected as target analytes in this study. We optimzed analytical methods of PAEs in polymeric matrixes and quantitatively measured PAEs in plastic marine debris (n=32) and fresh plastics (n=31) using LC-TOF/MS. Plastic items commonly found on beaches and at sea as marine debris were considered as target samples. PAEs were detected in most of plastic samples analyzed with the concentration range of 0.12 - 70734 ng/g dry weight (d.w.) for plastic marine debris and 0.03 - 15434 ng/g d.w. for the fresh plastics. Among three PAEs, DEHP showed the highest levels in both marine debris and fresh plastics. Except for some samples, the levels of PAEs were relatively high in plastic marine debris compared to those in fresh plastics, implying their potential absorption from surrounding environment. The present study provides quantitative information about phthalates contained in marine debris and their new plastics. This may be useful for understand environment exposure to anthropogenic chemicals through plastic pollution. Keywords: Phthalate esters, Plastic marine debris, Fresh plastics, Quantitative analysis

TH215

Weathering of plastic materials and identification of their leachates

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At the ocean surface the plastic is exposed to sunlight, oxidants and physical stress leading to degradation [1]. It is important to identify and assess potential environmental hazards from chemicals liberated from weathering plastics. The aim of our study is to develop a laboratory protocol for degradation of plastic materials and identification of their leachates. Pristine PE – polyethylene (x50 = 314μm) and PET – poly(ethylene terephthalate). (x50 = 110μm) were separately suspended in water (Milli-Q) and exposed to UV lamps of different intensities and subsamples were taken at different time points. We monitored the weathering of plastic materials in two ways; by measuring the infrared absorbance spectrum of the plastic materials themselves, and by the analysis of free chemicals leaching from the materials into the water. The plastic material was analyzed by FTIR spectroscopy before and after aging. An increase in intensity was seen for bands corresponding to the C-O and C=O bond, which is typical for the oxidation during the degradation process. Additionally, we conducted non-target analysis of chemicals liberated from the plastic materials. 25 μL of the water was directly injected into a UHPLC-HRMS instrument using a C18 column and a Q Exactive Orbitrap HF mass spectrometer (Thermo Scientific). For ionization both ESI and APCI were applied, and measurements were done separately in positive and negative ionization mode. The MS was run in full scan (100-1000 Da) at a resolution of 120.000 (at 200 m/z) with a data dependent MS/MS scan of the five highest peaks present in the full scan. Peak detection and alignment of the LC-MS data was done using Compound Discoverer 2.0 (Thermo Scientific). Degradation of plastic polymers can lead to low molecular weight polymer fragments and the formation of new functional end groups, especially carboxylic acids. We detected 98 compounds in ESI negative mode, whereof most of them (35) were only detected in the PET sample. For ESI positive 220 compounds were detected with PET showing the most peaks (57). Using APCI we obtained 427 and 246 compounds in negative and positive mode, respectively. Most of the leachates were not be present in the MS/MS database mzCloud, or MS/MS information was lacking. Literature: [1] B. Gewert, M. M. Plassmann and M. MacLeod, Env. Sci Process. Impacts, 2015, 17, 1513-1521.

TH216

Investigation of the effect of plastic additives on the plastic-water partition coefficient of selected persistent organic pollutants (POPs) in batch tests

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The vast amount of microplastics (MPs) that stem from abandoned plastic products as well as lost plastic resin pellets, which are raw materials of plastic products, end up in the ocean. In order to determine the ecotoxicological effects of the MPs on marine organisms, one should be aware that persistent organic pollutants (POPs) may be enriched on the surface of MPs as they are traveling through surface water systems. To manufacture plastic products as well as plastic resin pellets, a variety of plastic additives are applied to resins to protect polymers during production processes as well as to enhance specific properties and end-use performance of polymers. These additives may have a certain degree of influence on sorption and enrichment of POPs on the polymer surface. The objective of this study is to investigate the effect of plastic additives on sorption behaviour of POPs on low-density polyethylene (LDPE). The LDPE samples tested here are LDPE pellets from different origins incl. “virgin” LDPE pellets from two different producers, “virgin” LDPE pellets from a producer but from two different lots, LDPE pellets with lubricant, LDPE pellets with colorants, as well as LDPE pellets produced from post-consumer products. A series of batch experiments are in execution to study sorption of polycyclic aromatic hydrocarbons (PAHs) on these LDPE with different origins. LDPE samples used for this study have been previously milled to the size of < 1.500 μm in diameter at CARAT GmbH (Bocholt, Germany). The progress of the sorption is controlled using High Performance Liquid Chromatography (HPLC). Adsorption isotherm is applied to determine PE-water partition coefficients (Log K_{PE-W}) of PAHs. In a first series of experiment, sorption of naphthalene, i.e., a PAH with two fused benzene rings, on LDPE pellets from two producers as well as two different lots from a producer was investigated. These artificially produced LDPE microplastics (< 1.500 μm) are placed in batch reactors with naphthalene (log K_{OW} = 3,35, water solubility 32 mg/L at 25°C) dissolved in water with different concentrations. The batch reactors are placed on a shaker during the experiment. The first results showed that the equilibrium was reached within 28 days. No significant difference in Log K_{PE-W} was observed among these LDPE tested in the first round. TU Darmstadt and CARAT are participants of an EU project “PLASTOX”, a consortium of a JPI Oceans’ Joint Action.

TH217

DDTs and PCBs in plastic pellets from São Paulo coast, Brazil

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Marine environment is globally threatened by the presence of microplastics (small granules of of diameter), including plastic pellets that are raw material used in manufacturing of a lot of products that provide humans well being. Those pellets can be released during their transport and float on the surface water, adsorbing contaminants such as persistent organic pollutants (POPs), and strand on the beach. In this study, plastic pellets were collected in 42 beaches of 13 cities, along the São Paulo coast, southern Brazil to analyse POPs, such as polychlorinated biphenyls and dichloro-diphenyltrichloroethane and its degradation products.

Before the analysis, pellets were separated by degree of discoloration and composition and POPs were analysed only in polyethylene (PE) yellowing pellets. The method of analysis involved the extraction in Soxhlet apparatus with n-hexane and dichlorometane and purification with alumina adsorption column.The identification and quantification of DDTs and other organochlorine pesticides and PCBs were carried out in gas chromatograph equipped with electron capture detector and mass spectrometer, respectively. The percentage of yellowing pellets among total samples collected was about 20% and the composition of yellowing pellets determined by Fourier transform infrared spectroscopy was predominantly polyethylene (~70-90%). The partial results of POPs showed predominance of PCBs followed by DDTs regarding pellet samples from central and northern coast. The amounts of those compounds showed relatively high concentrations compared with published data worldwide. Samples are still being analysed but it is possible to observe that the amounts of PCBs in the central coast, that is affected by waste disposal and large port and industrial complex, were higher compared to non industrialized northern coast. Those data was compared to previously published data of POPs analysed in pellets (a mixture of several degrees of discoloration) from the same area and to results from International Pellet Watch (yellowing pellets) website and showed similar concentrations. Previous data showed that the number of plastic pellets found in each beach and the amounts of POPs found in each sample is directly related to the proximity of industrial complex and disposal area. \n

TH218

Ageing of polystyrene microplastics - particle characterization and interaction with organic compounds

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Concerns about the presence of micro scale polymers (i.e., microplastics) in environmental system are growing. Although the so-called vector effect of microplastics for persistent organic pollutants on a global scale seems to be of minor importance, microplastics as sorbents might be more important for compounds that do not undergo long-range transport on their own. Understanding the interactions between microplastics and those contaminants remains thus essential for evaluating the materials' impact. Despite the number of sorption studies available for microplastics, there is little information on how ageing of the particles influences their properties and ultimately the sorption behavior. The large variability of organic pollutants makes the determination of sorption data for all compounds nearly impossible. This calls for the application of prediction methods, such as the correlation between sorption and the physicochemical properties of sorbates (e.g., Kow). Poly-parameter linear free-energy relationships (ppLFER) have been shown to be a promising tool as they incorporate the contribution of individual molecular interactions by properties of the sorbate and the sorbent in a single equation [1]. We applied an accelerated ageing procedure on polystyrene microplastics. The physical and chemical particle properties were extensively characterized. Sorption isotherms were experimentally determined using a diverse probe sorbate set covering various substance classes. The sorption data was used to derive a poly-parameter linear free-energy relationship (ppLFER) for sorption of aged polystyrene microplastics to investigate the contribution of individual molecular interactions to overall sorption. The results showed that the development of a ppLFER equation allowed a precise prediction of sorption of organic compounds by aged polystyrene. The contribution of individual molecular interactions to overall sorption was evaluated, by which the sorption properties of aged particles could be characterization using the ppLFER approach. [1] Abraham MH. 1993. Chem Soc Rev 22:73-83.

TH219

Direct and potential transference of organic pollutants from littoral plastics to seawater

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Plastics and plastic debris can access to the marine environment through direct discharges and transport from coastal areas (air, rivers, etc). In this study the transference of hydrophobic organic contaminants from different polymers (materials and origin) sampled in the sourrounding area of Mar Menor lagoon is estimated. The desorption of these pollutants from littoral plastics and microplastics to seawater was characterized for 24 hours and the total content of organic contaminants in these materials was also determined, as indicator of potential transference to marine environment. Plastics acts as passive samplers in the environment accumulating hydrophobic organic contaminants which are transferred to marine system once they enter in the seawater column. PAHs, current use pesticides and personal care products were found in littoral plastics. A significant part of those contaminants can be desorbed from plastics to seawater in the first 24 h, particularly for less hydrophobic compounds. However slower desorption take place for more hydrophobic contaminants which can be transported to longer distances sorbed on plastic materials.

TH220

Effect of the presence of biofilms on sorption/desorption of cadmium to microplastics in the aqueous phase

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Microplastics (MPs) are small (5 mm – 1 μm) plastic particles that are present in various aquatic environments, including freshwater. Concerns exist regarding the potential of MPs to affect organisms, and among these concerns is the potential that toxicants sorbed to MPs can be bioavailable to organisms. In real aquatic environments, microorganisms and exopolymeric substances (EPS) are associated with plastics creating an external layer of organic matter, a biofilm. In the case of MPs, the biofilm associated with the particles will likely affect the sorption/desorption reactions of toxicants. The objective of this study was to assess if the presence of a microbial biofilm associated with MPs [(polyvinyl chloride (PVC)] would affect sorption/desorption of cadmium to the particles. We first investigated the biofilm growth on PVC MPs (125 – 250 μm diameter) in freshwater obtained from a zebrafish (*Dania reno*) husbandry facility after growth periods of 24 h, 96 h and 168 h. The presence of a biofilm on PVC particles was quantified by separation of the biofilm from the MPs with sonication and then by measurement of the absorbance (570 nm) of stained biofilm with Crystal Violet in 0.5 nM EDTA. Subsequently, a biofilm was allowed to grow for seven days on PVC particles. Both pristine and biofilm-associated particles (1 g/L) were exposed (separately) to a 100 μg/L cadmium solution for 24 h. After the removal of the particles, zebrafish larvae (96h post-fertilization, hpf), were exposed to the resulting cadmium solution. Cadmium bioavailability was assessed by measurement of changes in metallothionein-2 (*MT-2*) gene expression using quantitative reverse transcription PCR (RT-qPCR) after RNA extraction from whole larvae. We verified our results based on zebrafish bioavailability tests with analytical chemistry by measurement of cadmium concentration changes in the aqueous phase with inductively coupled plasma mass spectrometry (ICP-MS). We anticipate that our results (ongoing data analysis) will contribute to a better understanding of the fate and effects of MPs and their co-contaminants within a more environmental relevant scenario.

TH221

Identification and quantification of micro-plastics in marine samples from 5 µm to 5 mm by FTIR and Raman microspectroscopy

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Million tons of plastic debris enter the oceans every year and persists in the environment because of its long-time stability. It is mostly caused by industry (microbeads in cosmetic products, cleaning agents and industrial incorrect disposed raw materials) and inappropriate disposal of waste. This waste is degraded to microplastics below 5 mm. The biological impacts of microplastics on marine ecosystems cause problems for animals, birds and humans. Microplastics are micro-sized particles of synthetic polymers in a size range from 5 mm down to a few microns. They have been observed in marine ecosystems worldwide. Because of their small size microplastics can be mistaken for food and can be ingested by a variety of organisms. Not only microplastics themselves, but also embedded additives , adsorbed toxic contaminants or associated potentially pathogenic microorganisms pose a potential risk for the marine foodweb. In the first instance reliable data about the occurrence of microplastics in marine environments and valid analytical systems are necessary for a risk assessment. FTIR and Raman Microspectroscopy are able to identify microplastics in marine samples distinctly on the basis of their chemical structure. On samples collected from the Baltic Sea we determine particles on filters and ascertain which of these particles actually microplastics are. Afterwards we identify the polymer and the particle size. The most identified polymers are polyethylene, polypropylene, polystyrene, PVC, polycarbonate and polyester. Evaluation of all microplastics sizes reveals that most of the particles have a size below 50 μm. A comparison between Raman and FTIR spectroscopy for microplastics identification in marine samples was performed. The results were compared with regard to number, type and size of identified microplastics as well as measurement time and spectra quality. Both methods are excellent methods to analyse smaller microplastics < 500 μm directly on filters. In principle, Raman microspectroscopy can identify microplastics down to 1 µm. Not only the number and size of microplastics detectable by Raman and FTIR imaging varies but also the polymer type. A combination of both spectroscopic methods delivers complete and detailed microplastics analysis. Detailed results are given in the presentation.

TH222

Freshwater sediment microplastic from an urban river in the west of Scotland

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Microplastic (MP; < 0.5 mm) pollution of freshwaters remains largely unknown. A study was conducted in the River Kelvin in Glasgow to profile the distribution of

MP in an urban river and calibrate techniques for extraction and identification of MP from sediment using density separation and light and electron microscopy. Suspected MP were present in sediment samples collected on two dates in concentrations of 113 and 346 items per kg of dry sediment in December 2015 and February 2016, respectively. Secondary MP, namely fibres were predominant, comprising >88% of total MP counts. Methodological considerations so far indicate that commonly used NaCl density separation protocols are a reliable method for separation of different size particles and densities but may lead to overestimation of MP due to flotation of other non-polymer microdebris. Visual characterisation with light microscopy may lead to misidentification of MP pieces, especially for non-polymer spheres, potentially overestimating contribution of primary sources. Elemental analysis using scanning electron microscopy (SEM) can be used to calibrate purification and visual detection methods and improve accuracy and relevance of results. This study is a part of a PhD project of the presenting author aiming to describe the behaviour of micro- and nanoplastics in wastewater treatment systems and receiving waters.

TH223

Comparative study of occurrence and spatial distribution of microplastics in surface sediments from The Persian Gulf

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The Persian Gulf is the third largest gulf and one of the most highly anthropogenically impacted regions in the world. Microplastics (MPs) in aquatic environments are an emerging contaminant of concern due to their ecological and biological consequences. However, to the best of our knowledge, little is known about the prevalence of MPs in the Persian Gulf. Ten sampling stations (5 stations in high tide and 5 stations in low tide) were selected along the Strait of Hormuz (Iran) that exhibited different levels of industrialization and urbanization and included a marine protected area (MPA). At each site sediments were collected and a two-step method was applied to extract MPs from sediments. This study addresses MP quantification and morphology to assess the abundance, distribution, and types of MPs. And, the need to examine where MPs tend to accumulate across the beach zone within this area. MPs were found in 100% and 80% of high and low tide samples, respectively. Across all sites, fibers dominated with films and fragments providing minor contributions. Polyethylene (PE), Nylon, and Polyethylene terephthalate (PET) were the most abundant polymer types of recovered MPs. The highest concentrations of MPs were found in those stations along the vicinity of highly populated centers and municipal effluent discharge, with statistically significant differences in MPs concentration between sampling stations ($p < 0.05$). And also, the result showed that MPs tend to accumulate in high tide surface sediments in compare with low tide. Nonetheless, MP concentrations suggest that most stations within the studied area of the Persian Gulf are not hotspots for MP pollution. This study provides a ‘snapshot’ of MP pollution and longitudinal studies are required to fully understand the plastic contamination in the region. Other questions remain open, including the transport and fate of the plastic particles in the aquatic environment.

TH224

Explaining the behaviour and removal efficiency of micro- and nanoplastic by different drinking water technologies

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A continuously increasing body of literature documents the widespread occurrence of plastic in different aquatic ecosystems. Methodologies applied to determine micro- and nanoplastic in the environment are still under development. Consequently, the knowledge about micro- and nanoplastic quantities and fate in freshwaters is limited. Nanoplastic has not been detected in environmental samples so far. However, recent experimental studies revealed the formation of nanoplastic as a consequence to UV exposure. Thus it can be expected that nanoplastic is also present in surface waters that are used for drinking water production. This could result in human exposure to these particles and consequent adverse health effects. Whereas research has been carried out on the behaviour of nanoparticles during drinking water purification, the behaviour of micro- and nanoplastic has not been studied yet. This study intends to determine the behaviour of micro- and nanoplastic during the various steps of drinking water purification and the extent to which these particles can be removed. Therefore, different drinking water technologies based on sorption, oxidation and size exclusion are tested. In particular we focus on flocculation, granular activated carbon, UV and reverse osmosis. Field flow fractionation, pyrolysis GC-MS, and micro-FTIR are applied as analytical techniques. The behaviour of polystyrene micro- and nanoplastic during bench- and pilot-scale studies will be presented. By this, we try to answer the question if human exposure to micro- and nanoplastic could occur via the consumption of drinking water.

TH225

Microplastics and heavy metal contamination in sea salts: a threat to food safety?

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Are commercial sea salts on the European market contaminated with microplastics and heavy metals? To answer this question fifteen brands of commercial sea salts were collected from Belgian supermarkets. White, grey and red salts were selected with different origins and varying extraction and processing methods. In the lab, microplastics were extracted from the sea salt by means of a hydrogen peroxide treatment to dissolve the organic matter and further identified and quantified by means of a stereo microscope after filtering. Trace heavy metal concentrations were determined by ICP-MS (Inductive Coupled Plasma Mass Spectrometer). As sea salts are in direct contact with seawater, they might contain several contaminants present in the marine environment, but also the extraction and food processing methods may lead to increased microplastics and heavy metal contamination levels in the sea salt. For most heavy metals, the World Health Organisation and the Codex Alimentarius Commission already established maximum safety levels in food graded salt. In contrast, microplastics are non-regulated emerging contaminants, with no food safety levels identified yet. Preliminary results of the analysis (ordered by the federal ministry for Public Health of Belgium) showed contamination with microplastics in the investigated food graded sea salts to varying levels, where the amount of microplastics seems to be influenced by the brine processing of the sea salt. Hg and Cd were present in concentrations below the detection limit (45 μg/kg sea salt). Pb and As exceeded the limits for some sea salt samples up to concentrations of 1 mg/kg and 0.1 mg/kg respectively. The relation between contaminant levels and the origin, extraction and processing method of sea salt need to be further investigated. Still, the results of this pilot study revealed that the use of sea salt may have consequences for human food safety.

TH226

Are microplastic particles entering the human food chain via fish meal animal feed products?

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Microplastics are found in the digestive systems of many marine organisms and consequent risks to human health are starting to emerge. Trophic transfer may occur when organisms, such as bivalves, are consumed in their entirety. The gastrointestinal tracts of fish are frequently removed prior to human consumption; however, fish meal is produced with whole individuals, including digestive tracts, and fish by-products. Fish meal is used to feed livestock, such as farmed fish, pigs and poultry. Between 9.2 and 36.5 % of fish sampled from different geographic locations were found to contain microplastics in their digestive system[1-3]. This raises the possibility of trophic transfer of microplastics or of harmful chemicals adsorbed to such particles when fish meal is fed to livestock. The analysis of fish meal may help to assess microplastic pollution in commercially less valuable fish stocks and – if the geolocation of the catch is known –the geographical variation in levels of contamination. Four to five different fish meal products will be analysed in triplicate. Organic material will be digested using an enzyme digestion method and the digest will be filtered. Visual inspection of the residual material will be performed: anthropogenic particles will be identified and further analysis of the type of microplastic is then planned using Raman spectroscopy. The results will improve understanding of the potential for microplastics to enter the food chain via fish meal. Concerns about trophic transfer via fish meal may be allayed if quantities of microplastics are minimal. Higher quantities would indicate a need for further research into the extent and consequences of microplastic contamination in fish meal. References (1) Davison, P. & Asch, R. Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. *Mar. Ecol. Prog. Ser.* **432,** 173–180 (2011). (2) Lusher, A. L., McHugh, M. & Thompson, R. C. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar. Pollut. Bull.* **67,** 94–99 (2013). (3) Rochman, C. M. *et al.* Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Sci. Rep.* **5,** 14340 (2015).

Increasing the relevance of toxicity assessment in LCA: in the need for a cross fertilization between RA and LCA (P)

TH227

Environmental Safety Check (ESC) demonstrates freshwater aquatic safety of cleaning products by means of 'projected' environmental safety ratios

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Many substances are used to formulate cleaning products and most of them are intended for down-the-drain disposal; therefore, freshwater aquatic eco-toxicity is a well-known environmental hotspot area. To demonstrate freshwater aquatic safety of ingredients, in the context of a broader sustainability scheme for detergents, the Environmental Safety Check method (Pickup et al, 2016) was developed by the European sector association (A.I.S.E.). This method calculates 'Projected' Environmental Safety Ratios (PESR) for each substance, applying the conservative assumption that each ingredient is used at the same concentration in all formulations of the same product category on the market. Hereby, the method provides safety reassurance well beyond the existing regulatory framework, which requires demonstrating ingredients' safety only in isolation and as currently used. To obtain the 'advanced sustainability profile' logo under the A.I.S.E. scheme, all the ingredients in a product have to demonstrate a PESR below 1. Otherwise, with current data availability and ESC's conservatively projected ingredient volumes, safety margins for some ingredients would not be shown to be adequately wide. As a result, the ESC method supports the identification of those substances and formulations which may be less sustainable in the future because of a poorer aquatic eco-toxicity profile or limited available safety data. Initially, PESR scores were not calculated for some widely-used substances where robust scientific risk assessments had been published, e.g. substances assessed in HERA (Human and Environmental Risk Assessment) or EU Existing Chemicals risk assessments, and for perfumes compliant with IFRA (International Fragrance Association) codes of practice. To allow the ESC method to be fully comprehensive so that its calculations and sustainability insights could potentially be used also outside the context of A.I.S.E. scheme, the tool is currently being expanded to integrate additional information on the above substances. The objective of these additions is to provide a more complete sustainability assessment for cleaning products in respect of aquatic toxicity based on risk, as an alternative to hazard-based (such as CDV) or LCA-based ecotoxicity evaluation schemes (such as Usetox).

TH228

Freshwater ecotoxicity as an environmental impact category in LCA - Findings of a cross-functional taskforce

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There is an increasing interest to obtain holistic assessments of environmental impact of chemicals, i.e. integrating aspects such as aquatic ecotoxicity, eutrophication, climate change etc. To that end LCA is seen as a key assessment method. The currently ongoing 'Product Environmental Footprint`(PEF) project by the European Commission has the objective to provide LCA-based metrics as a basis for distinguishing between products based on environmental impact and covers both fast moving consumer goods as well as durable goods. The extension of the traditional LCA methodology to include the ecotoxicological impacts of chemicals raises some fundamental issues both methodologically and conceptually with the accepted norm of using risk assessment to manage chemicals within industry and by regulators. Thus, ECETOC has established a task force to investigate the method for evaluation of ecotoxicity employed in the LCA context of PEF. In order to broaden the discussion and perspective the task force was assembled from scientists with background in LCA as well as scientists with a role of experts in RA. The aim of the task force was to: i) conduct a scientific investigation of the 'USEtox' method for assessment of aquatic ecotoxicity in LCA, based on a simple case study with a virtual down-the-drain product, ii) compare LCA and environmental risk assessment methodology, which both characterize human intervention on the environment and provide a basis for decision-making; and (iii) to provide guidance on the interpretation and scientific relevance of USEtox results in the context of chemical impact assessment and selection of chemical-based (manufactured) products. The task force has also discussed options that could move forward the discussion of the relevance and practical aspects of assessment of ecotoxicological effects in the framework of LCA, integrating the perspective of the RA experts. \n \n

TH229

A framework for contaminants in environmental assessment of organic waste recycling in agriculture

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Ever-increasing waste production has prompted the need for more sustainable waste management, from economic, environmental and human health perspectives, while increasing food demand exerts additional pressure on agriculture and other food production systems, which are expected to feed a growing population in more sustainable ways, under growing resource constraints. Agricultural recycling makes it possible to effectively and synergistically utilise organic waste (OW), but when studying the waste-agriculture nexus, the consideration of contaminants and their impacts on the environment and human health are of paramount importance. The choice of using raw OW materials, compost or digestate as fertiliser and soil amendment should be based on a comprehensive environmental and (health) risk assessment of their relative potential benefits and negative effects. Such negative effects, or impacts, are due to the presence of potentially toxic contaminants —such as trace (metal) elements (TE) and organic pollutants (OP)—, microbial pathogens and the emergence of antimicrobial resistance genes. Various frameworks, alone or in combination, are available to undertake such assessments. This study aims to synthesise the state of the art of these assessments, and suggests a combination of approaches that fully addresses the dynamics and fate of TE, OP and pathogens following application of OW to agricultural soils. We performed a literature review to identify shortcomings in existing frameworks regarding the consideration of contaminants, and subsequentially identify suitable models for overcoming the gaps between existing knowledge and current practice. Finally, we describe an ideal contaminant assessment framework for scenarios of OW recycling in agriculture. Such a framework would overcome the perceived gaps and shortcomings of existing ones, by combining and adapting mature approaches, frameworks, methods and models for collecting flow and substance inventories (including the dynamics and fate of contaminants), assessing environmental impacts and risks to human health, and comparing waste recycling scenarios. It thus combines data acquisition and scenario definition methods, material and flow assessment methods, as well as environmental and risk assessment models (focused on human and ecotoxicity) and finally an extrapolation strategy for regional upscaling of assessment results.

TH230

Assessment of toxicological thresholds based on legal limits from REACH regulation

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REACH is a regulation concerning the Registration, Evaluation, Authorisation and restriction of Chemicals in Europe. To identify substances of very high concern (SVHC), REACH has established limits based on physicochemical properties concerning their fate in the environment (persistence and bioaccumulation) and their effect (toxicity) in Human Health and Ecosystems. The evaluation of the fate and effect of substances is carried out independently for each physicochemical property. If at least one of the limits is exceeded, the production and use of a substance shall be restricted. However, this evaluation neglects some substance properties and the combinatorial effect of different properties in the potential toxicological impacts. The main goal of this research is to improve the toxicological assessment of substances by defining "virtual substances" based on the regulatory limits from REACH, which are incorporated in the life-cycle impact assessment method USEtox to calculate toxicological thresholds. A virtual substance is characterized by having one of its physicochemical properties on the limit to be classified a SVHC by REACH. The properties considered were selected based on the existence of regulatory limits: half-life in air ($t_{1/2}$ air), water ($t_{1/2}$ water), soil ($t_{1/2}$ soil) and sediment ($t_{1/2}$ sediment); octanol-water partition coefficient (k_{ow}); water solubility (Sol_{25}) and Effect Dose for human toxicity (ED_{50}). The calculation also includes the influence of other parameters required by USEtox to calculate the impacts. Results show that the parameters Henry´s law coefficient, water solubility, and vapour pressure, not considered in SVHC classification, have a significant influence in the toxicological impact. The comprehensive analysis of the toxicological thresholds also allowed for the identification of the most important parameters considered in REACH: k_{ow}; LD_{50}; and half-life equivalent to the emission compartment. The toxicological thresholds calculated can be used to (i) provide a reference for understanding the significance of the toxicological impacts, (ii) comprehend the possible effects of combining different physicochemical properties into potential impacts, (iii) provide a new tool to assist in the evaluation of safer toxicological alternatives for substances restricted by REACH; and (iv) present recommendations to improve, support, and facilitate the classification of substance of very high concern (SVHC) in REACH regulation.

TH231

Modelling species sensitivity distribution parameters for data-poor chemicals using a QSAR-type modelling approach

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Since the adoption of the Water Framework Directive (WFD) in 2000, protection and improvement of European water bodies by achieving a good ecological and chemical water status has been a major priority in European water policy. However, up until now, only 53% of all European water bodies met the ecological status criterion. Furthermore, for many water bodies the chemical status remained unclear, despite the fact that especially chemical contamination poses a big impact on aquatic ecosystems. Therefore, in 2007 the REACh-framework was adopted, aiming to identify environmental hazards of emerging substances. In environmental risk assessment, the species-sensitivity distribution (SSDs) is a generally used model for the estimation of these hazards in ecosystems, plotting a log-logistic function of the potentially affected fraction of species (PAF) at an increasing toxicant gradient. However, these curves rely on ecotoxicity data of multiple

species which are lacking in many cases. Toxicity of substances on a single species can be estimated using QSAR-models, based on the substances physico-chemical characteristics. However, by using this approach, for each species in the SSD a new QSAR-model needs to be derived. An alternative is to directly estimate the SSD-parameters (mean (μ) and inter-species variation (σ)), again using commonly measured physico-chemical characteristics (e.g. octanol-water partition coefficient or vapor pressure). In this study, we constructed a QSAR-like multivariate linear regression model to accurately estimate SSD-parameters for approximately 1500 substances based on five of these characteristics, making use of the eToxbase of the Dutch National Institute for Public Health and the Environment (RIVM). Eventually, these innovative models may be suitable in preliminary risk assessment of emerging chemicals. Multiple models were trained on acute LC_{50} and EC_{50} SSD-parameters and ranged in explanatory value from 65%-95% (μ) and 33% (σ) for complex models to 50% (μ) for user-oriented models. Although all models turned out to be impractical in estimating the SSDs inter-species variation σ, prediction of the SSDs inflection point has shown to be very feasible. Even using the more uncertain user-oriented model, estimated SSD-models of the 10% least toxic substances are very unlikely ($P < 0.001$) to exceed to top 25% toxic substances, implying that the model may be of big value in decision-making regarding prioritization of chemicals.

Development and validation of standardised methods and their use in regulatory frameworks (P)

TH232

Ecotoxicological testing of waste materials: State of the art and future developments

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In the European Union, the hazard properties of wastes have to be determined according to the Commission Regulation (EU) N° 1357/2014. However, this document does not specify how the HP 14 property (Ecotoxic) has to be tested and assessed. In fact, there are two, approaches for this task: 1. Evaluation according to the CLP-approach, which defines the rules for the classification of chemical mixtures. In that case, the hazard of a waste is calculated according to its chemical composition, based on summation of classified components. The main problem of such an approach is that waste samples usually contain many, often unknown chemicals. 2. Ecotoxicological testing of waste samples, using standard ISO methods originally developed for the assessment of contaminated water or soils. An early version of this approach has successfully been used in an international ringtest. Recently, a strategy that combines both approaches described above has been proposed. The waste assessment starts with the evaluation of existing information on the chemical composition and the summation method is carried out according to the CLP regulation. If sufficient data on the composition are available the assessment can be finalized after this step. Also when the waste is already classified based on partial data, no further assessment is needed. In all other cases ecotoxicological tests have to be performed. First an assessment of the ecotoxicity of the waste eluates using aquatic tests, has to be done. If the waste sample is not classified as hazardous based on its aquatic toxicity, the ecotoxicity of the solid waste material is tested using terrestrial tests. The waste is classified as hazardous or non-hazardous when limit values for hazardous chemicals (in step 1) of for toxicity (in steps 2 and 3) are exceeded or not. Based on experiences of 20 years of waste testing, a test battery has been selected, consisting of three aquatic and three terrestrial tests (per compartment one microbial, one plant and one invertebrate species). Using the approach described so far and using examples from our own research in France, Germany and Belgium we will show that this strategy of combining an initial CLP-classification with ecotoxicological tests as a second step is scientifically sound, practical and easy to perform. However, open research questions still remain (e.g. the identification of threshold values, or the consistency of this assessment with the List of Waste).

TH233

ISO/CD 19040-2 Water quality - Determination of the estrogenic potential of water and waste water - Part 2: Yeast estrogen screen (Arxula adeninivorans)

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Endocrine active substances can cause adverse effects on human health and aquatic ecosystems. As these substances can have cumulative effects on organisms, effect-based screening tools like *in vitro* bioassays are the methods of choice to detect and quantify endocrine activities of known as well as unknown mixtures. Such preliminary screening tools for environmental monitoring need to be accurate, precise and robust to give a reliable response whether endocrine active substances are present in a given sample. Within the framework of the standardisation process of ISO 19040-2 “Water quality – Determination of the estrogenic potential of water and waste water”, an international interlaboratory trial was performed to demonstrate the applicability of the *in vitro* bioassay Arxula Yeast Estrogen Screen (A-YES®) for the detection of estrogenic activity in different water samples. Thirteen laboratories from four countries analysed the 17β-estradiol equivalents (EEQ) in nine samples including a spiked and an unspiked surface water, a spiked saline water sample, a native influent and a native as well as a spiked effluent sample from a municipal waste water treatment plant, a native influent sample from a hospital waste water treatment plant, a spiked extraction sample and a negative control (field blank). The negative control was negative for all participants in all replicates, and 2 samples (unspiked surface water and native effluent sample) were below LOD (mean LOD 1.8 ng/L 17β-estradiol (E2); range 0.7 – 3.7 ng/L E2) for the majority of the participants. The remaining 6 samples were positive with mean EEQ between 6.3 ng/L and 20.4 ng/L. With these 6 samples altogether 141 measurements (mostly duplicate analyses) were conducted, of which 139 were positive (*i.e.* 98.6 %). Repeatability and reproducibility for the positive samples ranged from 7.5 % to 21.4 % and 16.6 % to 28.0 %, respectively. Our results are in concordance with precision data derived from LC/MS/MS methods. The results demonstrate the suitability of the standardised method as an accurate, precise and robust tool (1) for freshwater and salt water monitoring programs, (2) for the observation of sewage treatment plant performance with regard to elimination of estrogenic substances, (3) for the surveillance of environmental quality standards, (4) as a pre-screening step prior to chemical analysis for any kind of water.

TH234

Novel bioassays using Chlorella and Daphnia for rapid detection of water toxicity

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Bioindication and bioassay coupled with chemical analysis are widely used for environmental pollution monitoring. Despite many water toxicity bioassays become more rapid and simple they often are not provided by special equipment allowing to maintain required conditions for test organisms and to automate the measurement process. This abstract focuses on our latest developments in this field. The application of highly-productive alga Chlorella (*Chlorella vulgaris* Beijer) strain allows significantly reducing the duration of the analysis. The number of cells increases up to 500 times for 24 hours when algal stock culture is grown in a compact cultivator. The bioassay is conducted in a multi cuvette cultivator for 24 water samples where light and temperature conditions are maintained equals and carbon dioxide is supplied from the air. The toxicity is defined by the difference between optical density of algae grown in tested water sample and control sample. The test algae are exposed to the toxicants over a period of 22 hours. New bioassay corresponds to other methods using green algae in sensitivity to heavy metals. In order to maintain the required light and temperature conditions we developed special climate chambers for bioassay using crustacean daphnia (Daphnia magna Straus). Samples of water are placed in a special device, which maintains a constant concentration of oxygen for crustaceans during biological testing. Toxicological test with Daphnia is conducted for 48 hours under these conditions. We proposed the method for detecting the relative intensity of delayed fluorescence (RIDF) of Chlorella algae for rapid determination of water toxicity. The special fluorometer was developed to automatically analyze 24 water samples by means of RIDF. Water and waste toxicity bioassay using RIDF of Chlorella algae takes 1.5 hours. \n

TH235

A novel, efficient, and ecologically relevant bioassay method using multiple aquatic fungal species for fungicide ecological effect assessment

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Fungicide is used to prevent fungal plant pathogen, and it may have high toxicity to aquatic fungi, which play an important role in natural aquatic ecosystems. However, bioassay method using aquatic fungi have not been well developed. In the present study, we developed a novel, efficient, and ecologically relevant bioassay method for fungicide ecological effect assessment. Candidate test species were selected by considering the following 4 factors: (1) widely distributed and frequently observed species in natural aquatic ecosystems; (2) including a wide range of taxonomic groups; (3) available from public culture collections; (4) suitable for microplate test method. Finally, we selected the following 5 fungal species: *Rhizophydium brooksiaum* (Chitridiomycota) strain NBRC 103829, *Chytriomyces hyalinus* (Chitridiomycota) strain NBRC 102555, *Tetracladium setigerum* (Ascomycota) strain NBRC 102389, *Sporobolomyces roseus* (Basidiomycota) strain NBRC 10566, and *Aphanomyces stellatus* (Oomycota, fungus-like microorganisms) strain NBRC 103817. An efficient test method using above fungal species was developed based on algal microplate assay, which is adopted in standard method of ISO 8692 (Water quality — Freshwater algal growth inhibition test with unicellular green algae). Test vessel was 96-well white microplate and test duration was 48 h. Fungal biomass was determined by ATP based luminescence method, which is adopted in standard method of ISO 13629-1 (Textiles — Determination of antifungal activity of textile products — Part 1: Luminescence method). The ATP luminescence is known to be proportional to live cell density and can be determined using microplate reader. Test performance was evaluated by conducting bioassays of 3,5-dichlorophenol and malachite green as standard test substances.

TH236

Transformation of organic chemicals in environmental fate metabolism studies: A comparison between aquatic sediment (OECD 308) and surface

water test systems (OECD 309)
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Among a broad range of laboratory environmental fate test methods, higher-tier assessments include metabolism studies in aquatic sediment systems under aerobic and anaerobic incubation conditions (OECD 308) and aerobic degradation/metabolism studies in natural surface water both with and without suspended sediment (OECD 309). In the natural environment, during and after application of crop protection agents, drift to water bodies with (suspended) sediment present is most prevalent. Hence, a possible influence of sediment on the transformation of organic chemicals in environmental fate metabolism OECD 308 and OECD 309 studies has been investigated, which involves evaluating the degradation results of a significant amount of crop protection compounds being subjected to both study types. One objective has been the screening of test compound patterns for the potential formation of degradation products in significant amounts in OECD 309 test systems, while not being formed in OECD 308 test systems, in which sediment is present. The second purpose has been to examine the influence of sediment on the test compounds degradation velocity. The results provide an insight into the effect of sediment on the degradation pattern and velocity of plant protection products in water/sediment when compared to water only test systems.

TH237
Comparison of Statistical Approaches for Quantal Data from Ecotoxicity Studies
J.W. Green, DuPont / Dynamic Simulation, Control, and Optimization
Quantal data is a type of count data that often arises in ecotoxicity study. A fixed number of subjects are exposed and the interest in is the number of those subjects that respond, e.g., die or develop a specific type of lesion or condition. There numerous ways to analyze quantal data. Alternative methods need not provide the same answers. In some cases, the differences can be very large and it is important to understand the limitations and applicability of various approaches that have been proposed in order to make a scientifically sound choice. Both regression and hypothesis testing methods are explored. For NOEC determination, the tests include the Cochran-Armitage test, with a Rao-Scott adjustment in case of overdispersion, and Williams' or Dunnett's test on data transformed to approximate normality. Recently, there has been much interest in applying generalized linear mixed models (GLMM) to quantal data using a binary error structure that accommodates overdispersion where needed. Williams' beta-binomial tests is an older approach that is a specific way to implement GLMM. GLMM models have good conceptual appeal and provide natural extensions to regression from which a percent effects level (ECx) can be estimated. A comparison of these tests will be explored through extensive computer simulation modeling and a substantial database of guideline studies the relative merits of these alternative approaches in terms of their power and sensitivity to find effects. For the regression approach, a comparison will be made of the quality of ECx estimates, in terms of precision, bias, uncertainty, and goodness of fit, from several versions of probit and logistic models based on normal and binary distributions that accommodate background incidence, data normalized to the control (Abbott's formula), familiar non-linear models based on an approximate normal distribution of (possibly transformed) within-treatment replicate proportions, and non-linear mixed effects (NLME) models for the mean response that use normal or binary error structure that accommodate overdispersion. Comparisons will be made from extensive computer simulations based on a large database of regulatory guideline studies. The choice of analysis has important risk assessment implications and it is important to understand the strengths and weaknesses of various approaches in order to make scientifically sound and practical choices.

TH238
Comparison of Statistical Approaches for Count Data from Ecotoxicity Studies
J.W. Green, DuPont / Dynamic Simulation, Control, and Optimization
There are two types of count data of that arise in ecotoxicity studies. The type of count data to be discussed here can take on a wide range of possible values that vary by individual subject. Examples include the number of eggs laid and the number of live young produced. Such count data are distinguished from quantal data, which are based on a fixed number of subjects that are exposed and the interest in is the number of those subjects that respond, e.g., die or develop a specific type of lesion or condition. Typically, count data have been analyzed as though they are continuous, often after a square-root transform. Advances in statistical methodology and software have made it possible to use a generalized linear models (GLMM) and nonlinear mixed effects (NLME) models approach using a Poisson, gamma-Poisson, or negative binomial error structure. In doing so, it is possible to accommodate overdispersion in a direct manner. This approach has intuitive appeal and has recently attracted much interest in ecotoxicology. Both NOEC and regression approaches to data interpretation are considered. For the NOEC approach, the power of various tests to find effects will be explored through extensive computer simulations and through a large database of guideline studies. Results from GLMM models with Poisson error structure will be compared to the previous standard approach of approximating a normal error structure on (possibly transformed) replicate counts and applying Williams' or Dunnett's test or applying a non-parametric alternative using the stepdown Jonckheere-Terpstra or Shirley test or a Dunn or Mann-Whitney pairwise analysis. For the regression approach, a comparison will be made of the quality of ECx estimates, in terms of precision, bias, uncertainty, and goodness of fit, from NLME models with Poisson error structure and gamma priors to accommodate overdispersion, to familiar non-linear models based on an approximate normal distribution of (possibly transformed) within-treatment replicate proportions. An application will be made to the recently issued mollusc reproduction test guideline. The choice of analysis has important risk assessment implications and it is important to understand the strengths and weaknesses of various approaches in order to make scientifically sound and practical choices.

TH239
Selecting non-toxic concentrations in bioassays
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Selection of the chemical no effect concentration is often needed in experiments with bioassays, for example for studying toxicokinetic processes or gene and protein expression. As there is no official guideline for choosing such a concentration, scientists can usually choose between statistically derived No-Observed-Effect-Concentrations (NOEC) and a certain modeled effect concentration (ECx). In order to deliver NOEC values, one could consider the variability of the control samples or, e.g. for dynamic assays, look for the measured chemical concentration which causes an effect not significantly different from zero. However, scientists generally have to choose the "non-toxic" concentration (NtC) case by case. Such selection is often based on the modeled dose-response curve, quality of the measured data, and the aim (e.g. to be above the limit of quantification (LOQ) available for chemical analysis), but mostly on personal experience. To overcome this ambiguity, we propose an algorithm for choosing a chemical concentration which can rationally be claimed to be non-toxic. This algorithm considers: (i) the modeled dose-response curves, (ii) all of the measured data, (iii) the statistical difference from effect zero or the control and (iv) the maximum effect that should not be exceeded by the confidence intervals. We have compared our approach with NOEC values and other modeling approaches and tested it based on many chemicals and different testing objects (different cell lines, zebrafish embryos) obtained from several studies. In a study on the cytotoxicity of 34 chemicals to the RTgill-W1 cell line, all our NtCs were between EC0 and EC5 and they were in agreement with the concentrations that we would choose based on experience. Additionally, 80% of these concentrations were above the LOQs reported in that study. For half of the remaining 20%, even EC50s would be below the limit detection. Thus, our algorithm can often be used without further improvements of the quantification methods. Finally, our approach is more conservative than existing approaches that are based only on the modeled dose-response curves, and more reliable than NOECs, which in some observed cases would cause an effect > EC30. By providing the algorithm code in different programming languages and developing a simple to use web application, we believe that our method can successfully be used by other scientists working with bioassays.

TH240
Luminescent bacteria testing in natural samples - inter laboratory comparison test
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Luminescent bacteria tests using *Aliivibrio fischeri* are standardized and are widely used methods in ecotoxicity testing of water, soil and sediment. Test laboratories ensure the quality of their methods with reference substances and proficiency tests but usually the sample in proficiency test schemes is an artificial clear liquid sample dissimilar to typical sample types like sediment, sewage water, and industry process water. Finnish Environment Institute SYKE organized an interlaboratory comparison test providing a coloured samples extracted from creosote contaminated sediment n addition to an artificial water samples spiked with 3,5-dichlorophenol. For the statistical analysis of the results, the mean or robust means from of individual EC_{50} values of the results of the participants were used as assigned values. Evaluations of the performances were done by comparing the results of each participant to the assigned values using Di%-values. The results were found to be in the same order of magnitude regardless of the different methods or equipment used. The difference between the participants was smaller in clear sample results while results from the coloured samples varied more. The maximum differences in EC_{50} values were 47 % and 167 %, respectively. Differences between various methods and equipments were small due to small data sets, but some trends were detectable. Kinetic methods with both tube luminometer and plate reader gave slightly higher EC_{50} values than standard method in both cases. The nature of sediment sample was suitable for comparison of kinetic luminescent test to the standard method. However, heterogenity of natural samples cannot be avoided and thus may be responsible for some of the variability of the results. Therefore in

future comparison tests more effort to find a homogenic sample and more thorough testing of the homogeneity of the samples will be done.

TH241
Influence of the peat particle size on the toxicity of boric acid to the reproduction of Folsomia candida in laboratory test with OECD artificial soil
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Theoretically, particle size and surface area have a reverse relationship. In the context of toxicity testing, the bigger soil particles have smaller surface area for the binding of toxic substances leading to higher bioavailability and thus, higher toxicity. However, no study has taken into account the difference of the peat particle size (PPS) in the OECD artificial soil. Here, we examined the influence of PPS on the toxicity of boric acid (BA) to the reproduction of Folsomia candida using OECD artificial soil. Our hypothesis was the bigger the PPS, the higher the toxicity of BA. The peat was sieved to obtain the small (≤1 mm), the medium (1< x ≤2 mm), and the big (2< x ≤3 mm) PPS. Three batches of soil with the only difference in the PPS were prepared and used to conduct separate toxicity tests, based on the OECD 232 (2009). The experiment was repeated twice. Our estimated EC50 values in increasing order of PPS in the first trial were 79.8, 59.2 and 51.2 mg/kg soil dry weight (sdw); and in the second trial the EC50 values were 97.5, 72.8 and 72.0 mg/kg sdw, respectively. However, pH of the test soils significantly decreased after 28 days. Therefore, we could not simply confirm our hypothesis. A further experiment focusing on the interaction between pH and BA was conducted. Many studies revealed their reverse relationship but have made no relation to the reproduction of F. candida. A 2x5 factorial design including pH and BA as factors was employed 1) to test the sole effect of pH and 2) to test the interaction between pH and BA to the reproduction of F. candida. The pH factor had 5 levels ranging from 4.5 to 6.5; the BA factor had 2 levels including 0 and 100 mg/kg sdw. Without BA, the mean values of juveniles in decreasing order of pH were not significantly different. However, with the presence of BA, they were significantly reduced at 95 % confidence interval. In conclusion, under laboratory conditions, increased PPS has an indirect negative impact on the reproduction of F. candida by decreasing soil pH. The results are discussed in respect of the resulting variation when following the OECD protocol.

TH242
Hazardous waste classification - tests methods for inorganic substances
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EU regulations for the hazard classification of chemicals and waste share the same hazard statements and make use of similar or identical hazard calculation (summation) methods for mixtures. It has been shown that the contents of metal compounds with ecotoxic properties often control the classification of waste. It has also been shown that for complex mixtures of contaminants, typically for wastes, the calculation methods are highly sensitive to the choice of metal speciation. Furthermore, important processes controlling the availability of toxic elements, such as species transformation and complexation on organic or inorganic sorbents in the waste matrix is not accounted for. The calculation methods for mixtures thus regularly results in misleading classifications which motivates the use of alternative test methods for assessing hazardous properties. Waste and chemical hazard assessment share the same legislative framework and ought to share the same test methods provided under the CLP. There is however little or no experience of applying them for assessment of waste. Regular standardised leaching tests intended for risk assessment have frequently been applied in spite of their unclear status in relation to hazard assessment. Waste often has a very variable composition, which requires assessment on a case by case basis. The objective of this study was to compare three frameworks for hazard assessment: (i) calculation (summation) methods for mixtures, (ii) methods for oral bio accessibility (BARGE and EN 14997) (iii) and leaching tests for ecotoxic availability (OECD nr 29 and EN 14735). Ash from incinerated waste was used as a model material. Preliminary results show that geochemical modelling is a powerful tool to support hazard assessment and development of robust test methods. Standardised tests intended for risk assessment proved to severely underestimate the hazard associated with the presence of toxic elements in the waste, while simultaneously falsely indicating hazardousness due to the presence of non-hazardous elements (e.g. Na, Ca and K). A set of test methods customized for inorganic substances and suitable for waste hazard assessment is suggested, providing more reliable results than the currently applied methods for waste. Compared to the more laborious test methods provided under the CLP the suggested test methods are simplified but robust enough to provide improved opportunities for assessment on a case by case basis.

TH243
Improvement of the ISO 10634 guidance document for the preparation and treatment of poorly water-soluble organic compounds for the evaluation of their biodegradability in aqueous media
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Current regulations on chemicals in most regions of the world recommend or require the assessment of biodegradability. It is commonly agreed that the assessment of biodegradability starts with a ready biodegradability test. To perform these tests, the recommendations of the ISO standards or OECD guidelines should be followed. Nevertheless, relevant infomation is missing when the chemical under test is not soluble in water. Since 2011, L'Oréal has worked on the preparation techniques in order to incorporate this type of compound in the media before the test. The systematic review of ISO 10634 standard dealing exclusively with this subject, was found as a good opportunity to disseminate the work carried out in this context. The recast of this standard is based on the updating of the methods already described as well as the proposal of new adapted methods. These bioavailability improvement methods in ready biodegradability tests were discussed to AFNOR and ISO working groups in order to revise the document ISO 10634. The performed experimental work made it possible to propose new bioavailability improvement methods and an subsequent update of ISO 10634 standard. This revised standard will make available to CROs all the information needed to improve the assessment of the readily biodegradability of water-insoluble chemicals. If the modification of an existing standard may seem long (several years), this must be put into perspective given the necessary scientific and bibliographic work and discussion between experts within the ISO/TC 147/SC5/WG 1. A new version of the 10634 ISO standard is expected to be published in 2018.\n

Environmental risk assessment of biocides: regulatory requirements, challenges and consequences (P)

TH244
A proposal for a risk envelope approach to assesses product families and Union authorisation under the Biocidal Product Regulation
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The purpose of the Biocidal Products Regulation is to improve the free movement of biocidal products within the Union while ensuring a high level of protection of human, animal health and the environment. The approach is based on, first the approval of the active substance (inclusion in the Union list) and afterwards the authorisation of products that contain such active substances. The BPR includes the possibility for companies to authorise a group of biocidal products as a biocidal product family (BPF) under the condition that all biocidal products within a BPF should have similar uses and the same active substances albeit at different concentrations, but such differences must not adversely affect the level of risk or significantly reduce the efficacy of the products. The BPF may consist from two to tens of products with different formulations, different uses and different product types increasing enormously the complexity of the risk assessment process and the way information is presented and communicated to all stakeholders. Once a product family is approved, applicants can add new products within the agreed boundaries of the family by a simple notification which needs to be made 30 days before placing the product on the market. It is foreseen that while probably reducing the number of individual product assessments, the BPF increases the complexity of the assessments and may create a heavy workload, mostly at the beginning of the process. Therefore approaches must be developed to keep the workload of regulators and applicants at an acceptable level and to make the risk assessment, risk management and authorisation of products transparent, comprehensive and consistent. The risk envelope approach is intended to do this, based on worst case scenario showing a safe use. Within a group of products of a family for each of the relevant risk assessment criteria (e.g. type of use, application rate, number of applications, concentration of active) a given use that represents the worst case is included. This identification of worst case scenario would then facilitate reviewing and assessing new uses or new products where the risk has already been covered in the BPF and is lower than the one assessed. This poster presents the challenges when assessing BPF and a proposed methodology based on risk envelope approach to overcome them.

TH245
Optimizing environmental risk assessment in a large biocidal product consortium
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Submitting a single biocidal product authorization dossier can be quite complicated and expensive. In order to assist companies in this aspect, biocidal product consortia are being set-up to decrease the financial and regulatory burden on

industry. Sodium hypochlorite, also known as bleach, has a wide variety of uses, and is used in professional and non-professional areas. Due to its broad application spectrum, a lot of sodium hypochlorite containing biocidal products and manufacturers are on the market and are seeking authorisation. Companies have decided to join forces and to develop a common biocidal product family containing sodium hypochlorite biocidal products used in product type 1-5 (Disinfectants). The current running consortium consists of 28 companies and 290 products. The large number of products and different companies, makes setting-up a biocidal product family structure challenging. An automated database was set-up to group products in metaSPCs (subgroups), which is in first instance based on the classification. Linking composition, uses and classification gives us the opportunity to effectively and efficiently perform a structured data gap analysis and environmental risk assessment. After identifying the environmental exposure scenarios, products need to be selected which contain the highest sodium hypochlorite concentration for that application and in addition, products containing substances of concern which might be hazardous to the environment need to be included. In such a large consortium, performing an adequate risk assessment will largely depend on the database structure in place, to identify the products which might entail the highest risk to the environment.

TH246

Challenges of application of environmental risk assessment methodology to PHMB

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A prospective and comprehensive environmental risk assessment (ERA) must be performed on the active substance for the market authorisation of biocidal products. The technical guidelines are made publicly available by ECHA. The data required for the ERA include the determination of physical-chemical properties (solubility, n-octanol/water partition coefficient), fate properties (adsorption/desorption, route and rate of degradation, bioconcentration), short-term and long-term ecotoxicity. PHMB is a synthetic mixture of oligomers with a huge range of molecular weight, positively charged (polyelectrolyte) at environmental pH. It exhibits acute aquatic toxicity below 1 mg/L and low toxicity for sediment and soil. The level of CO2 formation in degradation studies is low and formation of NER is observed. The present work focuses on the application of the ERA methodology to the difficult substance PHMB. The challenge associated with the application of the methodology are: i) to deal with the NER formation for Persistency assessment ii) to assess the hazard of PHMB and to determine reliable PNEC values iii) to model the exposure of environment since equilibrium partitioning method is of limited meaning for PHMB (mixture, no desorption). Since extractions techniques are often used to provide indication of the bioavailability, it does not seem consistent to consider PHMB as meeting simultaneously P and T criteria due to its very particular behaviour. With such difficult substance, the standard test regime should be adapted to allow consideration of non-standard data. We propose a Tiered approach. Tier 1 environmental risk assessment, which assumes 100% of bioavailability of the assessed substance and ignores the formation of non-extractable residues (NER), a half-life of 1000 days is a worst-case value. This assumption of 100% bioavailability leads to an overestimation of the environmental risks. Tier 2 environmental risk assessment is supported with further data on the nature and strength of the interaction of PHMB to solid particles, further data demonstrating that interaction with solid particles renders PHMB no longer "active", further data demonstrating that non-extractable residues (NER) are of low/no concern. The more relevant and reliable data are, the more robust is the environmental risk assessment. The purpose of this work is to initially review the challenges with the ERA of PHMB, then to highlight the need for more flexibility in testing strategy.

TH247

Challenges and solutions for the environmental exposure assessment of PT18 biocides.

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The registration of biocidal products according to the Biocidal Product Regulation (BPR), requires the assessment of possible risks of their use for the environment. For this, the emission of active substances (AS) and any other relevant substances of concern (SoC) are estimated based on Emission Scenario Documents (ESD) for the intended uses of the biocidal product. Standardized scenarios are developed for the most common uses of the different product types (PT). These scenarios are intended to describe a realistic worst-case situation, and are useful for screening level evaluation on the ecotoxicological risk of the product. However, default values used in these scenarios are often conservative, resulting in an over-estimation of exposure and hence risk for the environment. Consequently, risk assessments are to be further refined, based on the specific uses and application methods of the product, and the characteristics of the AS and SoCs. Here, different options are discussed for further refinement of the environmental risk assessment of insecticides in PT18. Although these products are not intended as 'down-the-drain' products, deposition of insecticidal products on the floor and other hard surfaces can be picked up during wet cleaning and as such be evacuated with the wastewater. A first assessment of the environmental risk demonstrated that particularly water and sediment compartments are very sensitive to the exposure of AS commonly used in insecticides. Therefore, several refinements are proposed, both at the level of the AS and at product level. These can be implemented at different stages of the emission pathway of a biocidal product: application, wash-off and degradation. As such, a more realistic estimate of the amount of product actually released in the environment during use of the insecticide can be obtained.

TH248

Comparison between US and EU active substance registration for environmental safety - Pyrethroids as a case study

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Active substances are designed to exert an efficacious dose to target organisms whilst minimising potential effects to non-target organisms. Therefore one of the key purposes of registration is to ensure environmental safety prior to marketing in products. The regulations in the EU are defined under the Biocidal Products Regulation (BPR) for biocides and the Plant Protection Products Regulation for pesticides used in agriculture. The BPR then further separates different biocidal actives by the type of intended use or product type. In the US, the Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) is responsible for evaluating and approving the registration of pesticide products. Three categories of pesticides (conventional chemicals, biopesticides and antimicrobials) are evaluated by three divisions within OPP (Registration, Biopesticides & Pollution Prevention and Antimicrobials, respectively). The process in the EU and US uses a similar framework for environmental risk assessment (ERA). The general approach is to compare the predicted environmental exposure concentration with the concentration of the chemical which is shown not to cause an effect on non-target organisms. The EPA and the BPR both use standardised ecotoxicity studies (E.g. OECD test guidelines) to determine the effect concentration. However, other details of the ERA are markedly different. For instance under the BPR the registrant is responsible for performing an ERA using a representative product and clearly defined worst case exposure scenarios and therefore carries the obligation to show environmental safety. In contrast, in the US the EPA conducts their own ERA on all representative uses of the active substance using very different exposure models. Additionally the assumptions and uncertainty factors that are considered in regard to the effect data are also divergent. Furthermore factors such as the environmental compartments and trophic levels considered, endangered species, resistance and socio-economic benefits, are treated differently between regulations. Pyrethroid insecticides are included in the current reevaluation of registrations of pesticides in the US by the EPA and the ongoing authorisation system in the EU under the BPR. Therefore, this group of chemicals serve as an interesting mechanism to examine the consistencies and differences between the evaluation of environmental safety of active substances in the US and EU.

Poster Corner Abstracts

Future challenges in sediment toxicity testing for environmental risk assessment (PC)

MOPC01
Exposure of Plant Protection Products in Water-Sediment Systems Used for Toxicity Tests
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Sediment toxicity testing is gaining an increasing importance within the scientific community. Prominent test organism are Chironomids, aquatic insects, for which there are two types of chronic test designs using the same water-sediment test system. The first one is designed to assess effects by exposure via water and employs spiking of the water column (OECD 219). The second is proposed for effect assessment due to exposure via sediment and employs spiking of the sediment (OECD 218). Because the same test system and organism are used one might assume directly correlated results from both test. However, inspection of effect endpoints from water- and sediment-spiked studies shows that there is no simple relationship as for example assumed by the equilibrium partitioning (EqP) method. The effect endpoints are normally derived from the initial concentrations in the spiked compartment. However, due to gradients between (overlying) water and sediment introduced by the way of spiking we must expect substantial temporal and spatial dynamics of local concentrations, especially in the vicinity of the interface beween water and sediment where the Chironomids are supposed to stay. For a test compound, exposure simulations with the mechanistic model TOXSWA were performed using study information and standard batch equilibrium sorption values for parameterisation (Koc ≈ 2000 L/kg). As initial concentration the respective EC_{50} were chosen The simulation results showed that sediment- and water-spiked tests develop typical spatial and temporal exposure patterns with the greatest dynamic at the interface between overlying water and sediment. In water-spiked tests, the penetration of sorbing compounds into the sediment is limited. Thus, average pore water concentrations over the full depth of the sediment appear not to be the best descriptor to derive effect endpoints when we additionally consider that the organisms live at or close to the interface. For the studies considered here, a similar exposure occurred in a few mm thick layer of the upper sediment for both modes of spiking with the respective EC_{50} concentrations. This shows that the water- and the sediment-spiked study may deliver generally consistent results if the assumption is made that the Chironomids inhabit a shallow sediment layer at the interface to the overlying water. In order to confirm these findings such assessments are foreseen for additional compounds with different sorption properties.

MOPC02
Pore water - The relevant exposure pathway for sediment risk assessment?
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In 2015, European Food Safety Authority (EFSA) issued a scientific opinion on environmental risk assessment for sediment organisms. This scientific opinion provides approaches on how to derive regulatory acceptable concentrations for sediment organisms and exposure to active substances and their transformation products, and how to link them in a tiered approach to predicted environmental concentrations for the sediment compartment. A list of uncertainties in relation to such approaches is given: Among them, EFSA acknowledge the need for understanding the differences between OECD and US guidelines and the possible consequences on toxicity estimates. However, despite some uncertainties, it is proposed in the scientific opinion to express the PECsed and RACsed estimates in terms of (1) total sediment concentration based on dry weight, normalised to either the OC content in the dry sediment or to standard OECD sediment with an organic matter content of 5 %, and (2) the freely dissolved PPP fraction in pore water. Our objective is to challenge the assumption that pore water is a relevant route of exposure for sediment organisms. Information was gathered from different sources such as: results of studies with Chironomids performed according to both OECD 218 and 219 guidelines, practical experience of these tests, and modeling with TOXSWA. For the comparison of the spiked water and spiked sediment test results, it was assumed that if one compartment was the relevant route of exposure, the ratio of the concentrations measured in this compartment from both test design would be similar to the ratio of the toxicity endpoints. This assumption could not be validated: for 11 substances there were no similarities whatever compartment was considered; for 1 substance, similarity was observed with overlying water concentrations ratio. The top layer of the sediment and the interface with overlying water are the key locations in this system, not only beause of the material exchange over time but also because Chironomids reside at this interface in the test system. However, for practical reasons, the analytical measurements of the substance concentration in pore water or in sediment in the OECD tests cannot be accurate estimates of the concentrations to which Chironomids are exposed. Therefore, toxicity endpoints expressed on these concentrations should not be considered reliable and are, at best, questionable in their use in the risk assessment.

MOPC03
Evaluating the Relative Sensitivity of Endpoints Generated During Midge Life-Cycle Sediment Toxicity Tests for US Pesticide Registration
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Midges (Diptera: Chironomidae) are frequently used as model organisms for evaluating hazard associated with sediment-bound test materials. In the United States, many scientists rely on the guidance document “Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates” (EPA 600/R-99/064). This document, which is now in revision, was developed by the US Environmental Protection Agency (US EPA) and originally released in March 2000. As currently written, procedures pertaining to life-cycle tests with the midge detail the collection of survival-based endpoints for larval and adults, as well as sublethal endpoints including larval growth, adult emergence (total/percent, cumulative rate, time to first emergence, time to death, and sex ratio), and reproduction (time to oviposition, mean eggs/female, egg cases/treatment, and egg hatchability). High variability in control responses and redundancy of information gained from similar observations brought about questions concerning the utility and/or value of certain endpoints for defining biological effect thresholds. Sediment toxicity testing with benthic aquatic invertebrates became a conditional requirement for pesticide registration in the US on October 26, 2007. This conditional regulatory requirement has led to the generation of data from life-cycle tests with midge conducted using consistent methods and according to GLP. Compilation of this data can therefore provide a unique perspective of overall test performance and endpoint sensitivity, which could inform future directions in study design. Streamlining the number of measured endpoints to those which are sensitive to stressors would improve the efficiency of the test method and also provide researchers with greater confidence that observed effects are indeed related to test material exposure and not merely a result of natural biological variability. In 2015, members of the Crop Life America Sediment Toxicology Subteam compiled a database of the results of these sediment tests. The purpose of this presentation is to review findings culminating from the review of this database to provide recommendations concerning how the methodology of life-cycle tests with midge (*Chironomus dilutus*) may be advanced.

MOPC04
Recommendations to Improve, Harmonize and Standardize test designs for acute and chronic sediment toxicity tests with Hyalella, Chironomid, and Leptocheirus.
H.O. Krueger, Wildlife International / Aquatic, Plant and Insect Toxicology
There has been considerable research in recent years to improve tests for Hyalella, Chironomid, and Leptocheirus, especially for water quality criteria and pesticide registration. Efforts to improve tests have focused on diet, water quality and using known age organisms for testing. Optimizing these parameters have improved performance in survival, growth, and reproduction and in doing so have allowed refinement of test endpoints. Much of the research has focused on freshwater species that include *Hyalella azteca* and *Chironomus dilutus*. There is a great deal of similarity in test designs and systems used for freshwater species with tests run in water baths having two exchanges of overlying water per day, however, saltwater tests have been run in different test systems and as static renewal studies. The 10-day acute tests for Hyalella and Chironomus have organisms that are fed during the test, are run with a photoperiod of 16h light:8h dark, and have survival and weight as endpoints in the test. The 10-day *Leptocheirus plumulosis* study is quite different since organisms are not fed, are under a 24 hour continuous light regime, and survival is the only endpoint. These 10-day tests should have a standardized photoperiod, all tests should have growth and weight as endpoints, and organism should be fed during the test. We also have data to support that the lessons learned from the Hyalella chronic study should be applied to Leptocheirus, such as the amount of growth that occurs during the test and expressing reproduction as number

of young per female. Uniformity in test design and test endpoints will allow more direct comparisons among the three tests. Such comparisons can be used to help prioritize testing. For example, if all three acute tests run, is it necessary to perform chronic tests on all three species, or only the most sensitive species.

MOPC05
The use of 10 d sediment-spiked toxicity tests with benthic macro-invertebrates and the insecticide lufenuron as a Tier 2 effect assessment approach
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A new EFSA scientific opinion suggests that for insecticide exposure in sediments the Hazardous Concentration to 5% of the test species (HC5) derived from a Species Sensitivity Distribution (SSD) constructed with 10-d sediment-spiked toxicity tests with benthic arthropods and the application of an assessment factor (AF) of 15 can be used to derive a Regulatory Acceptable Concentration for sediment organisms (Tier-2 RAC_{sed}). In order to evaluate the validity of this Tier-2 approach an outdoor sediment-spiked microcosm experiment together with several indoor sediment-spiked laboratory trials were conducted with lufenuron by using the same field-collected sediment. Lufenuron is a benzoylurea insecticide that targets chitin synthesis in arthropods. Once entering the aquatic environment, lufenuron quickly partitions to the sediment and may persist there, making sediment toxicity tests most appropriate. The aim of the present study was to generate a Tier-2 RAC_{sed} and compare this with the previously obtained Tier 1 RAC_{sed} and Tier-3 RAC_{sed}. To this purpose, we conducted 10 day toxicity trials, following a modified ASTM format, using several sediment associated arthropods. With the results obtained we constructed a SSD, derived an HC5, and derived a Tier-2 RAC_{sed} value. Valid 10 d sediment-spiked tests were performed with the benthic arthropods *Chironomus dilutes*, *Chironomus riparius*, *Caenis* sp., *Ephemera danica*, *Sialis lutaria*, *Hyalella azteca*, *Gammarus pulex* and *Asellus aquaticus.* The LC50 values obtained were used to construct an SSD.The calculated HC5-value of 1.82 (0.46-3.70) μg ai./g OC together with an AF of 15, resulted in a Tier-2 RAC_{sed} value of 0.12 μg ai./g OC. This Tier-2 RAC_{sed} seems to be protective and worst-case when compared with the Tier-3 RAC_{sed} of 0.263 - 0.395 μg ai./g OC derived from the outdoor sediment-spiked microcosm test. In addition, this Tier-2 RAC_{sed} was more than a factor of 2 higher than the Tier-1 RAC_{sed} (of 0.049 μg ai/g OC) that was also based on toxicity tests with field-collected sediment.

MOPC06
Incorporation of sediment specific aspects in the CRED evaluation system: recommendations for sediment ecotoxicity data reporting
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The development of environmental quality standards (EQS) for surface water monitoring and predicted no effect concentrations (PNECs) for marketing authorizations are based on effect data from ecotoxicity studies. In most of these frameworks, ecotoxicity studies used for risk assessment must undergo an evaluation of reliability and relevance. REACH defines reliability as “the inherent quality of a test report or publication relating to preferably standardized methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings”. In 2011, the European Chemicals Agency defined “relevance” as “the extent to which data and tests are appropriate for a particular hazard identification or risk characterisation”. In the same year, the United States Environmental Protection Agency published guidelines for screening, reviewing, and using literature toxicity data in ecological risk assessments. Available European guidance documents do not provide detailed information on how to evaluate reliability and relevance of a study. The Klimisch et al. (1997) methodology was used in many regulatory procedures where reliability needed to be evaluated. In recent years, a new evaluation method, the Criteria for Reporting and Evaluating ecotoxicity Data (CRED), was developed to strengthen the transparency, efficiency, and robustness of environmental hazard and risk assessments of chemicals, and increase utilization of peer-reviewed aquatic ecotoxicity studies for substance evaluations through improved reporting. Although many of the evaluation criteria are easily applicable to sediments, the methodology was conceived for aquatic toxicity studies and requires adaptation for its implementation in the evaluation of sediment ecotoxicity studies. We will propose a methodology for the application of the CRED system to assess the reliability and relevance of sediment ecotoxicity studies, which can be applied in the context of EQSsed and PNECsed development. We will address metal specific issues according to the most recent literature as well as criteria to help assessing relevance and reliability of mesocosm and field data using a weight of evidence approach when available.

Poly- and perfluoroalkyl substances (PFASs): Recent developments, sources, transport, fate and toxicity (PC)

MOPC08
Seasonal variation of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain)
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The contamination of the aquatic environment by perlfuoroalkyl substances (PFASs) becomes a problem since they are easily transferred between environmental compartments reaching groundwater, soils, sediments and biota. However, the partitioning mechanism and their fate in the environment are still not well known. Whereas the occurrence in the aquatic environment has been assessed by different authors, up to now little is known about their seasonal fluctuation and their influence in coastal and highly productive areas and fragile ecosystems. These ecosystems can be highly affected by human activities since they receive urban sewages and other by-products of human activities. The main objective of this study was to assess 13 PFASs in the Ebro Delta region and the surrounding coast in a total number of 87 waters, 71 sediments and 55 fishes in 3 seasons, in order to gather information about their occurrence, distribution, behavior and seasonal fluctuations in a Mediterranean estuary environment. Among the 13 PFASs, perfluorocarboxylic acids were the ones found at the highest concentrations in water samples. The most contaminated samples were influent and effluent WWTPs where perfluoropentanoic acid showed the highest concentrations in all sampling campaigns (100 to 2000-time greater than other water samples). In the case of sediments, perfluorooctanesulfonate (PFOS) was the compound detected at higher concentrations (< 1.02 – 22.58 ng/g dw). Nonetheless, perfluorooctanoic acid (PFOA) was the most ubiquitous compound in both matrix types. Waters showed a constant level of PFASs over the year with an insignificant decrease during winter and a subsequent increase during spring-summer. In contrast sediments showed a progressive decrease from autumn to summer. For seawater fish samples, the most detected compound was PFOS despite the stop in its production. These results agree with its high persistency, bioaccumulation and biomagnification. On the contrary, river fishes showed higher PFAS concentrations (ΣPFAS from 63.76 ng/g ww to 938.21 ng/g ww). In addition, perfluoroalkyl carboxylic acids were more concentrated than sulfonates. The bioaccumulation factor calculated for freshwater fishes denoted the high capacity of perflulrohexanoic acid, PFOA, perfluorononanoic acid and PFOS to be accumulated in fish from the surrounding waters.

MOPC09
Comparison of analytical methodologies for analysis of Poly- and perfluoroalkyl substances (PFAS) in Water
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Poly- and perfluoroalkyl substances (PFAS) have been discovered to be ubiquitous in the environment. These compounds have highly desirable properties that make their use in industry and manufacture essential. However, PFASs are also extremely persistent, bioaccumulate and potentially toxic to humans and wildlife. Traditionally, the perfluorocarboxylic acids (PFCAs)and sulfonic acids (PFSAs) have been the most studied classes and as a result the two most commonly used homologues perfluoro octanoic acid (PFOA) and perfluoro octance sulfonate (PFOS) have been regulated or phased out in the EU, US and other parts of the work. Consequently, alternate clases of PFAS have been used as replacements and their occurrence, fate and toxicity is less well known. This study developed a single analytical method for 31 PFAS using LC-MS/MS. The PFAS classes analyzed included the PFCAs, PFSAs, fluorotelomer sulfonates (FtSs), fluorotelomer alcohols (FtOHs), perfluorosulfonamides (FOSAs), perfluorosulfonamidoacetic acids (FOSAAs) and others. When dealing with several different water matrices and with the continual advancement of analytical equipment, it can be a struggle for even experienced analysts to choose the best method of sample clean-up and extraction to get reliable, and robust data. Traditionally, labor intensive and time-consuming extraction techniques like solid phase extraction (SPE) and liquid-liquid extraction have been used for concentration of samples to achieve required detection limits for PFAS in the ng/L range. These methods require large amounts of sample (100-1000 mL) and solvents for extraction. Recently, the development of automated online SPE systems have given rise to the possibility of achieving similar detection limits with the use of just a few mL of sample while significantly reducing cost, labor and time of analysis. On the analytical side, newer mass spectrometers have produced a large increase in sensitivity. This has resulted in large volume injection of the aqueous sample with no or minimal pretreatment to be suggested as a possibility for analysis of these contaminants. In this study, a single LC analytical method for the analysis of 31 PFAS using on-line SPE, were compared to direct large volume injection and offline-SPE followed by LC/MS/MS analysis. Factors like method reporting limits and ion suppression were considered. Further, the type of water matrix, cost and time of analysis will also be discussed for

analysis of EOCs.

MOPC10
Fate and transport of perfluoroalkyl acids (PFAAs) in soil and groundwater
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PFAAs emission to the environment represents a potential risk to human health. Although, long-term global scale fate and transport models have been used to investigate potential trends in environmental levels, temporal trends and transports mechanisms of PFAAs are not fully understood. Due to high solubility in water, the behaviour of PFAAs in soil is important for environmental fate estimation and prediction. Fate and transport of PFAAs in soil were studied in an attempt to investigate the groundwater contamination in the Ronneby municipality in south Sweden. Vertical distribution and fluxes in sediment cores were studied to estimate PFAAs loading to groundwater. Observed PFAAs levels in sediment and groundwater were used to estimate exposure and to build a quantitative exposure model calibrated on PFAAs levels for humans. Associated with long-term epidemiological studies, the current investigation supports extensive monitoring of PFAAs exposure and health effects in the population of Ronneby. Solid phase extraction and liquid–liquid extraction methods coupled with LCMS were used to determine the PFAAs concentration in sediment, surface water, groundwater, and drinking water in the Ronneby municipality. Local hydrogeological data were input to the PFAAs transport modeling for the soil.

MOPC11
Electrochemical treatment of perfluorooctanoic acid and perfluorooctane sulfonate: application to groundwater treatment
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Electrochemical treatment of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) using a boron-doped diamond (BDD) anode was investigated in a series of bench-scale batch experiments. Current densities ranging from 3 to 50 mA/cm^2 were examined. Treatment in natural groundwater and at environmentally relevant PFOA and PFOS concentrations also was evaluated, and compared to results obtained in electrolyte and elevated PFOA/PFOS concentrations. Results showed that the presence of chloride, TBA, and components in natural groundwater had no measureable effects on the observed rates of PFOA and PFOS removal and defluorination for the range of current densities tested. Data also indicated that the shorter chain perfluorinated compounds (e.g., PFHpA, PFHxA, PFHpS, PFHxS) are amenable to treatment using BDD anodes. PFOA removal was greater than that of PFOS, especially at lower current densities.The limited adverse impacts of chloride, hydroxyl radical scavenger, and natural groundwater constituents on PFAS treatment suggests that BDD anodes hold promise as a viable treatment option for treating mixed contaminant groundwater plumes. While perchlorate was readily generated, biological treatment of the electrochemically generated water was effective in removing the perchlorate. Overall, these results suggest that electrochemical treatment of PFOA and PFOS using BDD anodes may be plausible in conjunction with other technologies (e.g., bioremediation for perchlorate) in natural groundwater systems. Acknowledgement This project was funded by the Strategic Environmental Research and Development Program (SERDP) Project #ER-2424. The results and conclusions are those of the authors, and do not necessarily represent those of the United States Government, and no endorsement of the described technology is implied.

MOPC12
Best Environmental Practices (BEP) guidance for the AFFF, textile and related FluoroTechnology industries
S. Korzeniowski, BeachEdge Consulting, LLC; T. Cortina, The Fire Fighting Foam Coalition; K. Schubert, The Chemours Company; F. Adamsky, Daikin Industries Ltd
This poster presentation provides an overview of Best Practice Guidance (BPG) for the use of Class B Fire Fighting Foams (AFFF) and Best Environmental Practices (BEP) for the textile industry. These Practices can be used by stakeholders to help them to dramatically reduce the potential environmental footprint when using AFFF to fight Class B fires and when processing fluorinated durable water repellent (DWR) products. The Fire Fighting Foam Coalition (FFFC) developed a multi-faceted guidance document that focused on five key areas: Foam Selection, Eliminating Foam Discharge, Containing Foam Discharge, Firewater Disposal and Foam Concentrate Disposal. These items will be reviewed in the poster presentation. With focus on wet processing textile mills and finishers, the 11 steps of the " Guidance for Best Environmental Practices (BEP) for the Global Apparel Industry: Including Focus on Fluorinated Repellent Products" identify practices to minimize exposure and environmental releases. In addition, the poster provides a tool for brands and retailers to ensure goods received are finished using Best Available Technology (BAT) and BEP principles. The purpose of these guidance documents is to encourage the full utilization of BEP and BAT principles, and ultimately ways to minimize waste and environmental releases of fluorinated products to help achieve both business and stewardship objectives.

MOPC13
New Mechanisms in Assessing and Managing Per- and Polyfluoroalkyl Substances? Current Needs and Our Recommendations
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Poly- and perfluoroalkyl substances (PFASs) are a family of over 3000 chemicals that have been used in various industrial and consumer applications since the 1950s. Since the end of the 1990s, there has been a steady increase in efforts to assess and manage PFASs. Consequently, multiple PFASs have been recognized as contaminants of high concern and control actions are thus in place to reduce the exposure to them on the national, regional or global scale. However, despite extensive time and resources deployed, research and control actions on PFASs are still limited to a small selection of PFASs, primarily perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), while limited available evidence suggests a similar need for concern for the other overlooked PFASs. In order to accelerate research and control actions to the whole group of PFASs in the next decade, here we lay out the current needs for and challenges in assessing and managing the group of PFASs as a whole and propose new mechanisms in addressing them. In particular, we call for a new coordination mechanism to understand the needs in both scientific and regulatory communities, identify common grounds and goals, and define roadmaps with key milestones to achieve such goals

Fate, risk assessment and management of natural toxins: state-of-the-art, challenges and future prospectives (PC)

MOPC14
Plant toxins as groundwater contaminants - do we need to care?
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Plants produce a large variety of bioactive natural compounds which may serve as "chemical weapons" to protect the plants against competitors, pests and diseases. Some are well known as food toxins, e.g. cyanogenic glucosides in cassava or sorghum, solanin and chaconin in potato, and lectins in beans. Also animals browsing on toxin producing plants can be severely intoxicated by e.g. pyrrolizidine alkaloids from ragworts and groundsels, ptaquiloside from Bracken, and cytisin from laburnum species. But can these plant toxins act as environmental pollutants in water reservoirs and eventually present a threat to the quality of drinking water? This presentation will provide examples of known common plant toxins, their sources, production and environmental fate, and it will identify and discuss the parameters most critical to exposure assessment for plant toxins. Both crops and wild plants produce toxins often in amounts of up to kilograms per hectar. These are released from plant to soil via wash-off, or from roots and plant litter, thereby presenting a continuous non-point source. The highly water soluble toxins are readily leached to drains and groundwater. The rate of transfer of the toxin from plant to soil, the rates of abiotic and microbial degradation process along the path from source to recipient, and timing of events with climate are critical factors for the final toxin concentrations in water reservoirs. Plant toxins comprise a wide range of chemical properties ranging from volatile low molecular weight compounds to high-molecular weight proteins. Within this group, low-molecular weight glycosides are of special interest due to their high aqueous solubility. Three examples of potentially problematic pollution sources from plants are given: Cyanogenic glucosides from clover, glucosinolates from rape, and ptaquiloside from bracken. All are glucosides, but while the first two groups of compounds are toxic due to their primary degradation products (hydrogencyanide and isothiocyanates), ptaquiloside is the direct toxin (carcinogen) after activation. While groundwater monitoring data for natural toxins are generally lacking, first measurements for ptaquiloside demonstrate its presence, with stabilities in groundwater of up to 6 months. Perspectives are made with respect to future monitoring, modelling and risk assessment of natural toxins as groundwater pollutants.

MOPC15
Establishing a Phytotoxin Database for Environmental Risk Assessment
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Phytotoxins are natural toxins produced by plants with widely varying molecular structures and toxic effects. Despite possibly high concentrations of natural toxins in vegetation, crops and freshwater, they are not yet commonly perceived as

environmental contaminants of possible concern. This far, environmental exposure and effect studies have only been conducted for a very limited number of phytotoxins, and systematic and larger monitoring campaigns are completely lacking. \n A crucial challenge is to systematically identify among the plethora of phytotoxins those that actually present a serious environmental and human risk. For this purpose, a database is needed containing detailed information about phytotoxins and their corresponding poisonous plants. To this end, we collect information from different disciplines (e.g., botany, veterinary science, food safety, human medicine and agriculture), and from different media (e.g., textbooks, compendia, web sources, scientific papers). Currently, the database contains information on the plants occurring in Switzerland, their spatial distribution, as well as the toxin identification, structure, physio-chemical properties (in silico predicted or experimentally determined), and, where available, toxicity data. Several important issues in the database content and setup are discussed. These include the selection of relevant toxicological endpoints and effect organisms, as well as the difficulty that phytotoxin fingerprints of individual toxic plants are often not fully identified. Furthermore, aspects related to the database quality, such as data source reliability and inconsistencies, and data gaps, completeness and redundancy are discussed. The required information content is also investigated to allow a broad usage of the database. In the future, the database can be used in environmental risk investigations, for example in the field of *in silico* modeling or in surface water monitoring. We hope that the developed tool can further be used in other disciplines dealing with phytotoxins, since their importance might grow in the future.

MOPC16

Carcinogenic ptaquiloside in stream water at base flow and during storm events

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The globally occurring bracken fern (Pteridium sp.) is widespread in the broadleaf forests of Denmark, and densely populates several vegetation types in the Americas, Australia and on the British Isles. Bracken is toxic to livestock when consumed, and a group of potent carcinogens have been identified, of which the compound ptaquiloside (PTA) is the most abundant. Ptaquiloside has been shown to be highly water soluble, leachable from bracken fronds and litter, and present in the soil below bracken stands. During storm events throughfall from the bracken canopy was collected as well. Stream water samples were taken as grab samples, while throughfall accumulated in glass jars set out below the canopy. Field blanks and fortified lab controls were included to ensure reliability of the analysis. Ptaquiloside concentrations were determined using LC-MS/MS after a clean-up using solid phase extraction. Results showed that PTA levels in the stream were highly dependent on precipitation, and was rising considerably during rain events, peaking at 2.28 μg/L, before quickly (

MOPC17

What is the aquatic toxicity of saponin-rich plant extracts used as biopesticides?

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Saponins are natural surfactants, found abundantly in various plant species. Saponin-rich extracts from quillaja and quinoa have been registered by US EPA as active ingredients in biopesticides. To use biopesticides safely, we need to know about their toxicity towards humans and the environment. If saponin-rich biopesticides are efficient against pests, they are most likely also bioactive in the aquatic environment against non-target organisms. The aim of the study was to determine the aquatic toxicity of saponin-rich plant extracts expressed by the Hazard Concentration protecting 95% of the species (HC_5), which was derived from species sensitivity distributions (SSD). SSDs of saponin-rich plant extracts were based on EC50-values derived from concentration-response curves from acute tests on 12 non-target aquatic organisms. HC_5 of quillaja saponins, tea saponins and quinoa saponins were 9.17, 0.54 and 38.59 mg saponin /L, respectively. There is a big difference between the these kinds of saponins in toxicity, indicating that the species specific type of saponin affects its toxicity, making “read-across” between saponins a dubious exercise. Environmental Quality Standards (EQS) are commonly used as an acceptable concentration for protecting the populations of water organisms from chemical stress. EQS of these saponins are 0.92, 0.05 and 3.42 mg/L using an assessment factor of 10 to HC_5. Referring to the usage of saponin-rich plant extracts, they could release concentrations of 0.5 ~ 1.2 mg quillaja saponins /L, 0.5 ~10 mg tea saponins /L and 0.7 ~ 10 mg quinoa saponins /L, respectively to the aquatic environment, by assuming a full field dose on 30 cm water. These values are close to or higher than the EQS-values, which means that they might pose a risk to the aquatic environment if not used cautiously.

MOPC18

Degradation of pterosin B in Danish forest soils

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Pterosins is a group of naturally occurring substances found in ferns. They have been ascribed health-promoting effects as well as anti-diabetic activity, especially in traditional Asian medicine. However, they are also metabolites formed by hydrolysis of a group of noxious natural toxins such as the carcinogens ptaquiloside, iso-ptaquiloside, ptaquiloside Z, caudatoside and ptesculentoside (‘the ptaquiloside group’). This group of substances, in particular ptaquiloside, have been thoroughly investigated in the Bracken ferns (*Pteridium* spp.), which are classified by WHO/IARC as ‘possibly carcinogenic to Humans’. Ptaquiloside and *iso*-ptaquiloside can be used as representatives for ‘the ptaquiloside group’. The compounds can leach from fronds and hence contaminate soils and drinking water resources. Pterosin B is formed naturally by hydrolysis of both compounds. Pterosin B is sorbed more strongly to soil materials than the mother-compounds due to the aromatic ring-structure of pterosin B compared to the aliphatic nature of ptaquiloside and *iso*-ptaquiloside. Hence, pterosin B is expected to have a slower turnover in the soil environment. It is therefore hypothesized that findings of pterosin B in samples of soils could indicate any past presence of ptaquiloside and iso-ptaquiloside hence making environmental risk assessment easier. Degradation of pterosin B was studied in Danish acid forest soils. Fresh soil samples from bracken-covered and bracken-free areas were incubated with pterosin B for 10 days, whereas sterile samples were incubated for 23 days. Pterosin B showed unexpected fast degradation with full degradation in top-soils within 5 days. No degradation of pterosin B took place under sterile conditions. Pterosin B degradation followed the sum of two-first order reactions. Half-lives were shorter for loamy sand (4hrs) than for sandy loam soils (28hrs). The study demonstrates that pterosin B cannot be used as indicator for ptaquiloside/iso-ptaquiloside contamination of soils. Pterosin B is microbially degraded. Soil materials having very low microbial activity such as aquifer materials could accommodate longer half-lifes, hence making pterosin B a possible indicator compound for ptaquiloside/*iso*-ptaquiloside contamination.

MOPC19

Presence of ptaquiloside in Bracken based food, food supplements and alternative medicine (this poster has been presented at 2nd Plant Toxin Conference in 2016)

L. Rasmussen, Metropolitan University College

Ptaquiloside (PTA) is a noxious toxin present in Brackens (*Pteridium* sp.) and a number of other ferns found Worldwide. Bracken ferns are classified by WHO/IARC as “*...possibly carcinogenic to humans*”. Consequently, Bracken and PTA are placed on the WHO/IARC urgency list of potential carcinogenic compounds/products to be re-evaluated in 2018 and many countries has enforced a ban on the use of Bracken in food, food supplements and alternative medicine. Denmark and the Netherlands are examples of such countries. Nonetheless, it is still possible to buy and import Bracken based products as these products are widely available at internet-based stores – and – of course – it is still possible to pick fresh Bracken yourself. The purposes of this study were to generate an overview of the use of Bracken and to unveil the availability and toxicity of Bracken based products for the European consumer. The study was based on a literature review in combination with internet search and visits to Asian shops in Europe and China. A selection of products were imported to Denmark and analysed for content of PTA and the main degradation product pterosin B (LC-MS and HPLC-DAD) at the Metropolitan University College. Bracken is widely used for consumption in Asia (e.g. Japan (*warabi*), Korea (*gosari/kosari*) and China (*juecai/jue cai*)). Different servings are made of the young fresh fronds (the croziers). Sometimes the fronds are raw, sometimes used as pickled products, or boiled/blanched. Dried and frozen products are also available. In Japan, dried rhizomes are used for making flour for the delicacy *warabi mochi* (a sort of sweet cake). Also, traditional blanching of croziers with wood ash or similar alkaline material are used to lower the toxicity. Bracken is also eaten in South America and in Africa, where it e.g. is used for traditional medicine in the Eastern and Southern parts. The native population in North America are also users of the young fronds. In Europe, Asian Bracken products are found in Asian stores and as food supplements or alternative medicine (homeopathic products – pills and extracts). The study showed that, a wide range of these products can be imported from Asian or North American web-stores. PTA and pterosin B were present in many products and in highly variable concentrations. Blanching of fresh Bracken was not found to eliminate PTA in contradiction to previous reports. The food authorities are advised to take these findings into account. \n

In situ measurement of nanoparticles (PC)

MOPC20

Mobility of metallic (nano)particles in leachates from landfills containing waste incineration residues

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Incineration of municipal waste and sewage sludge is becoming an increasingly popular option for the disposal of waste materials and energy generation. The incineration process can concentrate metals in the incineration slags deposited in landfills. Emitted leachates contain a myriad of salts and metals; some of them in (nano)particulate form. In this study we collected the leachate from a Swiss landfill for municipal solid waste incineration (MSWI) residues along with the slags deposited at this site (waste incineration bottom ashes and fly ash, sewage sludge incineration bottom ash) from which simulated leachates were prepared. Basic water quality analysis (pH, DOC, TSS, major ions) and natural, incidental or engineered particles suspended in the leachate were characterized by NanoSight (for general size range), serial filtration with ICP-MS analysis for element specific particle size quantification and TEM/EDX to visualize particle morphology and composition. Special priority was given to those elements that have engineered nanoparticulate counterparts (Ti, Zn, Ag, Cu, Fe and Ce) to give an indication of 1) the current concentration and form of these particles emitted from the landfill, 2) the potential presence of engineered nanoparticles already in the samples, and 3) trends in particle size (change) in the leachate from different slags to provide an indication on particle mobility. Zn, Ag, and Cu had appreciable concentrations associated with small particulate matter (nano and 0.1 – 0.45 ?m size fractions) in natural and laboratory prepared leachates, while Ti (nano)particles were most abundant in the landfill leachate. Multiple sampling dates suggested relatively steady particulate matter in the leachate for most elements, but analysis of differently aged bottom ash slags from municipal waste revealed differences with age, indicating the influence of slag weathering in metal mobilization. MSWI residues are inherently a complex mixture of stable and unstable materials that are subject to continuous and dynamic changes over time. Therefore, in this manuscript we placed an emphasis on understanding the geochemical processes that are associated with MSWI residue weathering and how this may dictate the likelihood of particulate metals leaching into groundwater.

MOPC21
Exploring the nanoparticle eco-corona evolution with surface-enhanced Raman spectroscopy
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A concerted research effort over the last decade has enabled us to significantly improve our understanding of the core chemistry of engineered nanomaterials (ENMs) as they are released into the environment. One of the current knowledge gaps in environmental nanotoxicology is in understanding the role of the ENM surface interactions at the molecular level, particularly in relation to the macromolecules and ligands acquired from the host matrix (the eco-corona). The eco-corona is hypothesised to consist of dynamic, layered structures that impart molecular functionality to the ENM but due to its diverse and complex nature its chemistry remains largely unknown. The overall aim of this project is to explore the applicability of surface-enhanced Raman spectroscopy (SERS) to investigate the eco-corona formation around ENMs, and to examine the role of the eco-corona on bio-nano interactions. In a pilot study, silver nanoparticles (Ag-NPs) with citrate stabiliser were exposed to a selection of molecules and equilibrated for up to 72 h, a typical time frame for freshwater ecotoxicity tests. The eco-corona structure and evolution around the Ag-NPs was monitored by SERS, UV-Visible absorption spectroscopy (UV-Vis) and Nanoparticle Tracking Analysis (NTA). SERS provided direct evidence of the molecular competition at the surface. Strongly interacting ligands (cytosine) displaced the citrate coating immediately upon exposure but led to Ag-NP instability at higher concentrations. Weakly interacting molecules (tannic and humic acids) required time to displace citrate but the Ag-NPs were stable for a longer time. This study was extended to examine the interaction of algal exudates with Ag-NPs in freshwater media. Without exudates, the Ag-NPs dissolved within 72 h: only water was observed in SERS, and UV-Vis and NTA corroborate the loss of the NP structure. When exposed to algal exudates, UV-Vis and NTA showed that Ag-NPs were more stable, however, SERS revealed that citrate remained on the Ag-NPs surface. This suggests that the exudates did not displace the citrate but instead formed a protective secondary layer around the primary citrate layer. These results demonstrate that SERS based methods have the potential to provide molecular information at the ENM surface, complementary to those accessible by UV-Vis and NTA. Such information can be utilised for design of safer ENMs by, for example, modulating the surface interactions to control persistence or rate of dissolution.

MOPC22
Development of methods for the detection of metallic nanoparticles in complex environmental and biological samples
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Metallic nanoparticles (NPs), such as silver (Ag) and copper (Cu), are highly incorporated into consumer goods, potentially creating risk at the end of the consumer-product life. Thus, environmental monitoring of NPs in aquatic environments is highly recommended. However, conventional elemental analysis techniques lack effectiveness when it comes to complex environmental and biological samples. Single particle ICP-MS (spICP-MS) provides precise measurements of size, number and mass distribution of metal-containing NPs, even at low predicted environmental concentrations. This study aims to develop methods of detection of NPs in complex environmental and biological matrices and compare this cutting-edge technique to conventionalICP-MS. Ag NPs (3) and base (TMAH) digested and diluted for total metal analysis and single particle analysis, respectively. Total metal analysis showed high metal levels coating the chorion (5797±962 ng Cu/embryo and 143±37 ng Ag/embryo in chorionated embryos), but did not accumulate in the embryo tissues (453 ± 69 ng Cu/embryo and 0.3 ± 0.04 ng Ag/embryo in dechorionated embryos) to the same extent. This study contributes to the development of methods to understand the behavior and distribution of these NPs in aquatic environments.

MOPC23
Mapping of accumulated Y/RhBITC-doped SiNPs and Cs in basil leaves by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS)
J. Ko, Korea Institute of Korea (KIT); Y. Hwang, KITOX / Future Environmental Research Center
Silica nanoparticles are well discussed nanoparticle in various fields. They have been shown good performances as sensor, catalyst, drug delivery and so far. In contrast, rapid consumption also suggest the problem as an environmental pollutant. For such reason, many researchers have been studying focused on the influences, especially based on the organisms in natural environments. For the purpose, plants are good study model since they are existed in the lowest level of a food chain. In here, we chose sweet basil (*Ocimum basilicum*) as the model plant due to its high consumption of cooking ingredient. As a pollutant exposed to environment, Y/RhBITC-doped silica nanoparticles, which have rare earth element of yittrium (Y) and rhodamine B isothiocyanate (RhBITC) dye doped in the core of silica nanoparticles, were applied for more effective monitoring. Since Si already highly contained in plants at concentration ranging from 0.1 to 10 %. The prepared nanoparticles of 165 μg mL^{-1} were exposed for 7 days, and laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was applied for analyzing a solid surface sample and detecting the accumulated nanoparticles with low concentration. To study the influences of co-pollutant, furthermore, we added cesium ions with 100 and 1,000 ng mL^{-1} in the nutrient solution as a secondary pollutant. And the obtained results showed with elemental distribution maps.

MOPC24
Quantification of pesticides in fungi and midge larvae in the presence of nanoparticles
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Natural and artificial nanoparticles are ubiquitous in the environment and might influence the environmental fate of anthropogenic trace substances by sorption-processes. Exposure studies of pesticides mainly focus on the dissolved fraction whereas in the environment many organic micropollutants tend to interact with micro- or nanoparticles. In this study the influence of nanoparticles, present in water and growth medium, on the bioavailability and effect of pesticides in biota is investigated. The uptake and toxicity of the fungicide propiconazole by the fungi *Laccaria bicolor* as well as of the insecticide thiacloprid on the midge larvae of *Chironomus riparius* in the presence of different nanoparticles is studied. Midge larvae were exposed to pesticides in the presence of zeolite and aluminum oxide nanoparticles. In case of fungi organosilica nanoparticles were used. The required exposure concentration of the pesticide in the medium was calculated based on previous sorption studies and was verified by HPLC-MS measurements. After the exposure time, of 96 h in case of midge larvae and 3 days in case of fungi, the internal pesticide concentration in the organisms was analyzed. Therefore, a liquid-liquid extraction method was developed based on the QuEChERS extraction procedure followed by quantification with HPLC-MS. With recoveries >60%, based on deuterated internal standards, the quantification in the low ng/g range was possible. Biota samples of only 1-30 mg wet weight were required for a single measurement. The measurements showed decreasing pesticide concentrations in the exposure medium with increasing nanoparticle concentrations. These lower pesticide concentrations correspond well with reduced effects observed in biological experiments. The results well reflected the incorporated concentrations analyzed in fungi and larvae.

New developments in ecotoxicology for the risk assessment of single and multiple stressors in insect pollinators: from the laboratory to the real world (PC)

TUPC01
Preliminary results from low-variability and low-uncertainty semi-field trials: A way towards an acceptable test-power
M. Wang, WSC Scientific GmbH / Dept Efate Modelling; A. Görlich, WSC Scientific GmbH
Recent field and semi-field trials conducted in the context of pesticide registration

have received substantial criticism by authorities. One key issue that has been put forward in practically all evaluations by authorities is the limited test-power of current trials that makes it impossible to detect small effects, e.g. differences of colony size of 7%, the threshold recently proposed by EFSA (2013). The resons why in practice this test-power is hard to reach are manifold. They include the uncertainty due to analysis of a limited fraction of a hive (e.g. number of cells), the use of visual assessments to measure colony strength or the analysis of foraging activity based on a relatively small number of foragers. But also the high variability introduced by current test designs is a reason for not achieving a sufficient test power, such as the large variability by conducting visual assessments of adults or site variability. In the present study, sources of uncertainty and variability were assessed to develop a modified field methodology and test design, which increases the test power of both field and semi-field trials. We show preliminary results to give insight into how much these methods increase test-power.

TUPC02

Sources of variability and uncertainty in honeybee field and semi-field trials and how to overcome them

M. Wang, WSC Scientific GmbH / Dept Efate Modelling; A. Görlich, WSC Scientific GmbH

Current field trials with honeybees are characterised by a relatively large variability and uncertainty between replicates, which results in a relatively low test power. In field studies variability between sites and between hives is a main obstacle for reaching a sufficient test power to detect effects of 7% as proposed by EFSA (2013). Furthermore, using current approaches (OECD 75, EPPO 170) uncertainty about the similarity of hives at the test start and also when monitoring the hives is very large. We therefore, explore the reasons for and quantify the amount of variability and uncertainty in field and semi-field trials in order to propose optimised study designs and evaluation methods for both field and tunnel tests.

TUPC03

Comparison of toxicity of selected insecticides to the red mason bee, Osmia rufa L.

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Among a huge number of pollinator species, such as honeybees or bumblebees, solitary bees (red mason bees) also play a crucial role in pollination of many plants, especially tree fruit crops. Red mason bee populations are likely to be exposed to residues of many chemicals including plant protection products (PPPs). However, there is still not enough data on toxicity of PPPs to both red mason bees and other non-*Apis* species. The aim of presented studies was to evaluate the lethal effects of eight insecticides on the adult red mason bee, *Osmia rufa* L. (Hymenoptera: Megachilidae) in an acute contact test. The tested insecticides that are commonly used in Poland were contained in different formulations: deltamethrin (EW), chlorpyriphos (EC), spinosad (SC), imidacloprid (SL), dimethoate (EC), bifenazate (SC), fluvalinate (EW), and chlorantraniliprole (SC). The endpoint of the laboratory tests was red mason bee mortality after 48 h of their topical exposure. Also, LD_{50} values were estimated. The results showed that the majority of the tested products was toxic to *O. rufa*. However, according to available literature data, their toxicity was much lower than toxicity to honeybees. Among all the evaluated insecticides, deltamethrin and chlorpyrifos showed the most adverse effects on the survival of the red mason bees. Bifenazate and chlorantraniliprole were the most selective to the bees.

TUPC04

A method for a solitary bee (Osmia spp.) first tier acute contact laboratory test: an update

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The recently updated EFSA draft honeybee Guidance document also specifies other hymenopteran pollinators, like solitary bees and bumblebees, as groups to take into consideration when assessing the risk of plant protection products to pollinators. However no validated test protocol and consequently no extensive data set is available to compare sensitivities of other relevant pollinators to those of honeybees. Within the current project of the ICPPR Non-Apis working group a start was made to develop a first-tier acute contact test for *Osmia* spp. bees. Based on the honeybee OECD213 and Ladurner et al. (2005) a test was designed using dimethoate as test substance, *Osmia bicornis* , *Osmia cornuta* were housed in groups and feed either with a wick-action or open device or a flower petal attractant. First results indicate that reproducible results were obtained using the open and wick-action devices. In these tests, control mortality was never higher than 13 percent. Furthermore, sensitivities of *O. cornuta* and *O. bicornis* appeared to be rather similar with LD50-96h values ranging from 0.8-1.3 and 0.4-2.3 µg a.s./bee for *O. cornuta* and *O. bicornis*, respectively. Indicating that a validated and workable test guideline is within reach.

TUPC05

Differentially expressed genes on brains of africanized Apis mellifera exposed to thiamethoxam

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The monoculture promoted the reduction of native areas in Brazil. In addition, as a result of the expansion of agricultural areas, there was an increase in the use of pesticides to control pests and pathogens. These xenobionts can also affect non-target insects, as the bees, important pollinators. Thiamethoxam is a neurotoxic insecticide of the neonicotinoids class commonly used in Brazil. This insecticide has a systemic action in plants, reaching pollen and nectar, resources collected by bees. In this way, this study aims to verify how thiamethoxam can affect the gene expression in the brains of africanized *Apis mellifera*. For this, 15 days old bees were exposed to a diet containing a sublethal dose (2 µg active ingredient / L diet) or a high dose (50 µg active ingredient / L diet) of thiamethoxam. For the control group, the diet was provided without insecticide. Individuals were collected 10 days after the beginning of food supply and the brains were dissected to perform RNA extraction using TRIzol (TRI Reagent) protocol. The transcriptome was made from the Illumina platform and qPCR was subsequently performed for analysis of differentially expressed genes. Fifty-seven genes showed differential expression between the control and exposed. Most of these genes encode short proteins with unknown functions and some are proteins involved with transcriptional, sensory and neuronal processes. Our data show that the insecticide can alter the level of gene expression in the brain of exposed bees. More analysis is necessary to uncover the biological role of these genes.

TUPC06

Simple modelling approaches to refine exposure for bee risk assessment based on worst case assumptions

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The risk assessment for plant protection products to bees has attracted a lot of attention over the past five years or more. Current estimates of exposure (e.g. EFSA, 2013) are based on 90th percentile concentrations of active substances present in pollen and nectar in the field. Although suitable for acute risks, in field concentrations are not suitable for chronic assessment especially for honey bees which feed from colony stores before making foraging flights or for larvae which are fed from in-hive food stores via nurse bees. Other areas of exposure such as to pollen and nectar in following crops or to guttation may also be better estimated by use of simple exposure models. We will present simple methods based worst case assumptions to model chronic adult and larval honey bee exposure to spray applications of plant protection products (PPP) which take into account in-hive storage of pollen and nectar and also approaches to model exposure levels in succeeding crops and guttation water. Case studies will be presented demonstrating how these worst case model exposure estimates can be used in refining the risk assessment for bees offering a robust, worst case and cost effective alternative to field studies. Having better, robust, modelled exposure estimates for in-hive food reserves can aid in the assessment of both single PPP stressors and interactions with multiple stressors (e.g. disease and Varroa mites).

TUPC07

Exposure of flower-visiting insects to pesticides in the European agricultural landscape

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Latest research on biodiversity in agricultural landscapes suggests that flower-visiting insects (FVI) such as wild bees and butterflies are not sufficiently protected from the effects of pesticides. To implement appropriate risk mitigation measures pesticide exposure of the relevant groups of FVIs has to be determined. Within the research and development project "Protection of wild pollinators in pesticide risk assessment and management" (FKZ: 3715 64 409 0; funded by the German Federal Environment Agency) exposure scenarios for FVIs were developed and exposure was quantified. We categorised FVIs by their habitat requirements which we derived from scientific literature and ecological trait databases. In our analysis we identified traits that influence exposure probabilities (e.g. flight season, mobility, flight time during day). These ecological properties also determine relevant exposure pathways for the different categories of FVIs, such as uptake of systemic pesticides by herbivore life stages of lepidoterans.Furthermore, we assessed the pesticide exposure of in-field and off-field habitats. We included all relevant exposure sources (e.g. spray application, seed treatment) and considered the major pathways of pesticides from in-field to off-field habitats. Finally, we synthesised those results to establish exposure scenarios for the specific FVI groups.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective (PC)

TUPC08

Using field data to support the derivation of an EQS for zinc

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Several studies have used quantile regression based approaches to derive or validate water quality criteria directly from datasets of collated chemical and ecological monitoring data. Similar approaches have been applied to current monitoring data from England and Wales which is used for water quality classification purposes under the Water Framework Directive (WFD). This approach is applied to assess the level of protection afforded by the UK Specific Pollutant EQS for zinc of 11 $\mu g\ l^{-1}$ bioavailable zinc. The present study follows a "limiting function" approach to define the bioavailable zinc concentration at which adverse effects start to become observable on the in-situ ecology. The statistical analysis is performed using quantile regression applied to high quantiles of the dataset. The diversity of the community is not adversely affected by zinc at levels below 88 $\mu g\ l^{-1}$ bioavailable Zn (EC10), although there is a limiting effect on the number of taxa at higher bioavailable zinc concentrations, with good ecological status no longer likely to be achievable at bioavailable Zn concentrations higher than 287 $\mu g\ l^{-1}$. Quantile regression analysis was used to identify any decline in the maximum achievable abundance of the selected taxa with increasing bioavailable zinc exposure. The ecological measurements are based on raw abundance data, and have not been normalised to take account of habitat conditions. Figure 2 shows the effect of zinc on the abundance of the crustacean family Asellidae, although another family of crustaceans, Gammaridae, showed no significant decline over the range of zinc exposures observed at field sites. Benthic invertebrate communities are adversely affected by bioavailable zinc exposures. There may be a reduction in diversity above 88 $\mu g\ l^{-1}$ bioavailable zinc, and sites with exposures of greater than 287 $\mu g\ l^{-1}$ bioavailable zinc are unlikely to achieve good ecological status for benthic invertebrate communities. No effects would be expected on the abundance of sensitive taxa if the proposed UK EQS for zinc of 11 $\mu g\ l^{-1}$ bioavailable zinc was complied with, although an exceedance of this threshold would result in a failure to achieve good ecological status through noncompliance with the UK Specific Pollutant EQS.

TUPC09

Bio-met: a user-friendly tool for bioavailability-based Environmental Quality Standard (EQS) assessments of metals

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The effects of metals in the environment depend on their bioavailability. For several base metals, biotic ligand models (BLMs) exist that allow the calculation of the bioavailable fraction of metals in freshwaters, taking into account the physico-chemistry of the water. Here, we present Bio-met, a user-friendly tool based on these BLMs. Bio-met allows the user to perform bioavailability-based compliance checks simultaneously for 4 metals using a simple Excel-based interface. The required inputs for the tool are the dissolved ($< 0.45\ \mu m$) metal concentrations, pH, Ca concentration, and dissolved organic carbon concentration. The latest Bio-met version includes three key improvements compared to earlier versions. The tool has been validated for a wider range of physicochemical conditions (notably at high pH). The user can now edit the reference EQS for the metals that are assessed as specific pollutants under the EU Water Framework Directive. Finally, in addition to Cu, Ni, and Zn, Pb has now been added to the tool. Bio-met is targeted to people involved in metals and water quality management, i.e. both industry and regulators. The tool can be easily applied in water quality compliance programmes as performed under the EU's river basin management plans. The tool can be coupled to databases allowing automated calculations of metal EQS compliance, integrating bioavailability. The application of Bio-met as a tool for compliance check and regional risk assessment of metals in freshwater will be presented.

TUPC10

How different can it be? Impact of region-specific methodology on the environmental quality standard of nickel.

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In 2013, an Environmental Quality Standard (EQS) was established for nickel (Ni), which is classified as a Priority Substance under the European Union's Water Framework Directive. The Ni EQS is derived from a process that includes aggregating bioavailability normalized data from chronic laboratory ecotoxicity tests into a Species Sensitivity Distribution (SSD). According to EU methodology, an Assessment Factor (AF) ranging from one to five is applied to the resulting HC5 from the SSD; in the case of Ni, an AF of one was chosen, such that the EQS is equivalent to the HC5. Using a reasonable worst case approach based on water chemistry parameters (hardness, pH, and dissolved organic carbon) that maximized Ni bioavailability, a reference EQS of 4 μg bioavailable Ni/L was established. In other jurisdictions such as Australia/New Zealand (ANZECC), Canada, and the USA, Ni is also classified as a substance requiring a numerical standard that protects aquatic life. Existing standards for Ni in these jurisdictions are based on older, acute ecotoxicity data, and account only for water hardness. Guidance among the different regions follows the same general approach, but region-specific differences occur with respect to data selection, endpoint selection, and the choice of SSD models. To determine the impact of the different regional standard determination methods on the magnitude of a nickel standard, we applied current regional guidance from ANZECC, Canada, and the USA to the accepted EU chronic ecotoxicity database. The resulting regional standards were analyzed to determine if harmonizing ecotoxicity databases among the jurisdictions would narrow the differences, or if other choices in the standard determination process (e.g., data extrapolation) are more important.

TUPC11

Use of TICKET-UWM as a Site-Specific Risk Assessment Tool

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The Tableau Input Coupled Kinetics Equilibrium Transport Unit World Model (TICKET-UWM) was developed originally as a screening-level model developed for a simplified one-dimensional, two-layer lake or impounded river. Screening-level models have been used principally in development of integrated rankings of chemical hazards that are based on environmental cycles, chemical behavior, and toxicity. For this purpose, the models have been applied to idealized environmental settings, or unit worlds, and have typically been used to evaluate a critical or maximum loading rate of chemical that could be released into the environment without causing an exceedance of a water quality criterion or some other toxicity measure. Although TICKET-UWM was developed initially for idealized environmental settings (i.e. the unit-world), development of the model over the last few years has resulted in a model that has utility in providing site-specific information on key metal risk assessment quantities (water column/sediment exposure concentrations, bioavailability, and metal fate/mass balance) under various loading scenarios and time scales all while explicitly considering site-specified water body attributes (e.g., settling rate, depth, hydraulic retention time, etc.) and site-specific water chemistry. Example risk assessment questions that can be addressed by the TICKET-UWM include the following: How do water column and sediment metal exposure concentrations and bioavailability change in response to variations in water chemistry? How do water column metal exposure concentrations scale with variations in loading? What is fate of metals added to the water column as soluble salts or metal concentrates. This work describes recent advancements on TICKET-UWM including the results of validation studies where the model has been used to describe field data and model enhancements to include WHAM VII and the effect of redox conditions on metal speciation in the water column and sediments.

TUPC12

Removal of Metals from the Water Column under Transformation/Dissolution Conditions for Chronic Hazard Classification.

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This work is aimed at adapting the Transformation/Dissolution Protocol (T/DP) to establish the rate and extent of partitioning of metals from the water column so that the data can be used in metals chronic classification. The UN GHS and the EU Classification, Labelling and Packaging (CLP) hazard classification schemes include the concept of degradation whereby rapid degradation from the water column (greater than 70% removal in 28 days) results in different classification cut-off values and categories. For metals and inorganic metal compounds, the rapid and irreversible removal from the water column can serve as a surrogate for the rapid degradation concept for organics, recognizing that metal ions can be made non-available from the water column by a number of processes. Three sediments were selected to represent a reasonable worst case (RWC), reasonable best case (RBC) and a sediment of intermediate composition focussing on the Fe and Total Organic Carbon (TOC) concentrations as both are considered to be important factors in metal partitioning from the water column. The RWC sediment has an Fe and TOC content of 1.6% and 1.2% respectively while the RBC sediment has an Fe and TOC content of 6.0% and 3.3% respectively. 10 g/L of sediment was added to pH 6 T/D solutions containing 1 mg/L Ni. The sediment loading was based on calculations of the settling flux of solids to the sediment in a typical lake environment. The 1 mg/L metal loading was selected as it is equivalent to the highest loading for chronic classification. pH 6 was selected as it is typically a worst case for metal dissolution. The solutions were sampled for analysis of the dissolved concentrations of Ni as a function of time. Results of the 96 h tests suggest that all three sediments could be suitable for achieving 70% removal of Ni from the water column in 28 days, with the intermediate case reaching 77% removal at 96 h. Additional 96 h experiments were conducted using the RWC sediment and pH 6 T/D solutions containing approximately 1 mg/L of Co, Cu, Pb, Zn and 0.1 mg/L Ag. Results indicate that >70% removal is achieved for Cu, Pb and Ag within 96 h and predicted to reach this point within 28 days for Co and Zn. An evaluation of Ni removal at pH 7 was also conducted to determine pH effects on metal removal rates. The T/DP adapted to measure the rate of metal removal from the water column offers an experimental resolution to the issue of chronic classification for metals.

TUPC13

Structural and functional response of river sediment microbial communities to environmental concentrations of copper and arsenic, alone or in mixture

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Sediments are an essential component of aquatic ecosystems both in terms of biodiversity and of ecological functioning. They receive direct or indirect inputs from the water column or from the watershed including some toxic and persistent contaminants (e.g. trace metals and PCBs) which can accumulate over time and impact exposed organisms. Among benthic organisms, microbial communities are major players in various key ecological processes such as organic matter recycling, greenhouse gas production and biomass production contributing to benthic food webs. However, knowledge about the effects of accumulated contaminants on the structure and functions of sediment microbial communities is scarce. In this context, the main aim of this work was to evaluate the structural and functional impact of chronic exposure to environmental concentrations of copper and arsenic (alone or mixed) on river sediment microbial communities. Natural uncontaminated surface sediments collected in a French River (Ain) were exposed for 21 days to Cu and/or As at nominal individual concentrations of 40 mg/kg in the artificial channels. The response of heterotrophic microbial communities to metals was evaluated both in terms of genetic structure (using ARISA analysis) and functional potential (using exo-enzymatic, metabolic and genetic analysis). A pollution induced community tolerance (PICT) approach was also performed to assess if the exposure led to an increase in the capacity of microbial communities to tolerate metals. Our results showed rapid (within 48 hours) and marked effects of Cu alone on the exposed communities. It led to a significant inhibition of microbial functions such as respiration and denitrification as well as beta-glucosidase, leucine aminopeptidase and phosphatase activities. Chronic Cu exposure also induced an increase in community tolerance to Cu, as observed by PICT measurement using beta-glucosidase activity. In contrast, the effects of As were mostly undetectable. Under mixture exposure, the effects were similar or higher than those provoked by Cu, depending on the measured parameter. All together those findings reveal that metals accumulation in sediments can impact exposed microbial communities thus affecting their functional role in aquatic ecosystems. It confirms the need for developing studies to better understand the ecotoxicological impact of contaminants on natural sediment communities and to improve risk assessment of metal contamination.

Challenges in Assessment and Management of Cosmetics and Personal Care Products (PC)

TUPC14

A framework for dynamic estimation of aquatic environmental concentrations of microplastics via WWTP discharge

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Down-the-drain exposure models provide a valuable screening-level tool for estimating environmental exposure to substances which are treated and discharged at municipal wastewater treatment plants (WWTPs). Microplastics enter WWTPs from a variety of sources. As such, exposure models traditionally used for chemicals may also be utilized for particle emissions into the environment from WWTP discharge. These models often account for removal in WWTP as well as in-river decay processes. However, in light of incomplete and changing knowledge on microplastic fate in surface waters, we developed a framework in which microplastic use rates and general properties can be used to estimate the range of expected environmental concentrations depending on assumptions about removal and decay. We developed a web-based tool incorporating 10 removal rates and 10 decay rates encompassing the typical and extreme ranges of possible values. Each of the 100 model runs produces a distribution of Predicted Environmental Concentration (PECs) representing each effluent impacted stream as described by the iSTREEM® model which estimates spatially-explicit concentrations of chemicals in effluent and receiving waters across the US. Output visualization in the interactive tool includes a broad view of all possible combinations in a matrix format, and a detailed view of the full distribution of PECs for individual model runs. Within the matrix, each of the 100 individual cells correspond to a selected percentile of the PEC distribution (e.g., 95th percentile) for tha combination of removal and decay. We demonstrate the utility of this framework using WWTP influent loadings of polyethylene microbeads from liquid soaps and shower gels estimated using per-capita usage (Gouin et al 2011) and combine with individual facility population served and flow estimates using the iSTREEM model. We can the investigate the question … *What kind of environmental concentrations might we estimate using these emissions*? This dynamic framework can be used to help inform environmental exposure assessments by readily providing PECs based on varying model inputs on WWTP removal and in-stream decay rates for microplastics, which continues to evolve as more research is conducted. While this framework was applied to the US at a national scale, the framework itself is not geographic-dependent and could function equally well utilizing PEC distributions from Europe or elsewhere.

TUPC15

Determination of negative effects of Methylparaben on sea urchin (Paracentrotus lividus) by embryo and fertilisation toxicity tests.

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First used as preservatives in the mid-1920s for pharmaceutical products, paraben use was rapidly expanded to food and cosmetics. Methylparaben is widely used in such industries like paint, paper and especially cosmetic, food and drug. Methylparaben is introduced to the aquatic ecosystems after usage and excretion just like all other chemicals. Methylparaben is present in almost all of the samples collected from rivers, lakes, sea, influent and effluent waters of waste water treatment plants, in range of ng/l-_g/l.Despite the high degradability in aquatic environment, the halogeneted degredation product of parabens are persistent and tend to accumulate in the sediment and organisms. The main concern arises from their endocrine disrupting and carcinogenic potential. There are scarce scientific lterature on the effects of parabens aquatic organisms while there are researches on test animals and human cell lines. In the toxicity tests for methylparaben, EC_{50} values were calculated as 11,2"_62 mg L^{-1} for water flea(*Daphnia magna*) that were exposed for 48 hours, 91 mg L^{-1} for green algae (*Pseudokirchneriella subcapitata*) that were exposed for 72 hours; 5.9–9.6 mg L^{-1} for *Vibrio fischeri*that were exposed for 15 minutes. However, the studies about the toxicity of parabens are mostly subjected to freshwater organisms, there are not sufficient data for the marine animals. There are the arising concerns that marine animals have potential risc about the endocrine distrupting effects since the precence of parabens in marine environment are revealed. In addition to that, there are no studies found that employed sea urchin (*Paracentrotus lividus*) as test organism, which is one of the important units in marine ecosystem. The larval stages of the sea urchin are known to be important and more sensitive to the to determine the effects of the pollutants such as metals , persistant organic chemicals, pesticides and pharmaceuticals. This study is aimed to determine the negative effects of methylparaben on sea urchin (*Paracentrotus lividus*) embryo so the concentrations were chosen higher than environmentally relevant concentrations. According to results, parallel to increasing concentrations of methylparaben decrease the fertilisation rate of *Paracentrotus lividus* and in the embryotoxicity test higher concentrations of methylparaben lead to increase of larval abnormalities compared with control group.

TUPC16

Dispersion, fate, and toxicity of aged titanium dioxide nanocomposite-based sunscreens in aqueous environments

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In the assessment and management of cosmetics and personal care products, sunscreens are of emerging concern regarding both human and environmental health. The fate and impact of mineral nanoparticulate UV-blockers, such as TiO_2 nanomaterials, is under continued consideration from a regulatory perspective due to their potential impact on both consumers and the environment. After leaving the skin either through bathing or everyday usage and cleaning, the nanomaterials contained in the sunscreen can be released into rivers, lakes, sea shores, and/or sewage treatment plants. The nanomaterial behaviour, fate and impact in these different systems is largely determined by its surface properties, (e.g. the nanomaterial coating type) and lifetime. The present work aims to develop the eco-design of sunscreens through the minimisation of risks associated with nanomaterials incorporated into the formulation. All stages of the sunscreen's life cycle must be considered in this aspect, from its manufacture to its end-of-life, through its use by the consumer to its impact on the exposed environment. Reducing the potential release and/or toxicity of the nanomaterial from the sunscreen is a decisive criterion for its eco-design. Different, relevant TiO_2 UV-blockers of varied size and surface coating (e.g., stearic acid, methicone, dimethicone) have been selected for this study. The UV-blockers were incorporated into a typical water-in-oil (w/o) formulation at different mass concentrations and the resulting sunscreens were characterised in terms of nanomaterial localisation, sun protection factor, and photo-passivation. The risk to the direct aquatic environment was assessed by evaluating the release of nanomaterials from the sunscreen through a simulated laboratory aging procedure. The size distribution, surface charge, and degradation state of the by-products, as well as their nanomaterial concentration and colloidal behaviour were determined in a variety of aqueous environments (e.g., seawater, freshwater). Ecotoxicity of the sunscreen by-products and their risk to marine organisms were assessed using coral symbiotes and tropical corals, evaluating both lethal and sublethal toxicities. The data dissemination and provided risk knowledge from the present work will help guide European regulations, provide better information for consumers, and allow for easier decision-making for manufacturers.

TUPC17

Fate of inorganic and organic UV filters

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In the European Union and Switzerland, 26 organic compounds and one inorganic (titanium dioxide TiO_2) compound are registered as UV filters. Some of the organic UV filters have shown hormonal activity in vivo. On the other hand UV filters have already been detected in the aquatic environment. To assess the importance of swimming pool waters as a source of organic and inorganic UV filters in surface waters, elimination rates of nano-TiO_2 and organic UV-filters in activated carbon or precoat filters from outdoor swimming pools have to be determined. The fate of UV filters was studied in four different outdoor swimming pools, and two sites at Lake Zurich and the smaller lake Katzensee. To monitor the inorganic UV filter TiO_2 water samples were collected and filtered through a 0.4 μm cellulose acetate membrane. After digestion of the filter samples were diluted by deionised water and Ti was quantified on an inductively coupled plasma optical emission spectrometer (CIROS, Spectro). Organic UV filters were preconcentrated by Oasis HLB solid phase extraction and afterwards determined on a high performance liquid chromatography coupled to triple quadrupole mass spectrometer. Monitored UV filters were currently used compounds in sunscreens which were as follows 2-Ethylhexylsalicylate (EHS), 2-phenylbenzimidazole-5-sulphonic acid (PMDSA), Avobenzone (AB), Ethylhexyl methoxycinnamate (EHMC), Octocrylene (OC) and Oxybenzone (OB). In the swimming pools and the compensating reservoirs Ti concentrations varied between 1 and 9 μg/L. After the precoat filter Ti concentrations were in the range of below limit of quantitation (LOQ) to 0.8 μg/L. The precoat filter acts as an efficient physical barrier for nano-TiO_2 particles.Regarding organic UV filters concentrations up to 24 μg/L of OC followed by PMDSA were detected in the compensating reservoir of Waldacher. The UV filters EHS and EHMC were found in concentrations ranging from below limit of quantitation (< LOD) to 1.5 μg/L. AB reached a maximum of 0.003 μg/L and OB was constantly around the LOD. A nearly complete removal of nano-TiO_2 from swimming pool waters by the filter systems was observed. In contrast, the precoat as well as the activated carbon filter were not able to quantitatively retain selected organic UV filters. Discharged waters from public swimming pools may thus substantially contribute to the load of organic UV filters in surface waters.

TUPC18

Ecotoxicity of natural complex mixtures: raw material of plant extracts which are resin-like solids. A follow-up and a conclusion.

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Under REACH review program several kinds of substances are considered: monoconstituents, multiconstituents, & UVCBs. Across these substance types several families always present a challenge to test especially in ecotoxicology and environment. One of these challenging groups is fragrances. \n Fragrance chemicals fall under multiple categories, they can be natural, synthetic, monoconstituent, multiconstituent or considered as UVCBs. One group of fragrances that falls under the title of multiconstituent/UVCB are known to be most difficult to assess: Essential oils. Essential oils (EO) are complex mixtures, with differing but often low solubility, high volatility and are known to contain some toxic constituents such as terpenes. Some of these substances are even more complex: gums, resinoids and concretes, sub-categories of essential oils. They are as complex as EO but their composition is mostly unknown and their physical state leads to further difficulties for ecotoxicity testing: they are (mostly) solid(ish), extremely viscous resins, with a frozen honey-like texture. Last year we presented protocol and results from a set of OECD 202 using WAF technology [1], and we observed no adverse effect below 100mg/L (loading rate). However, some effects appeared at the highest loading rate of 1000 mg/L. We could not conclude about it. We then decided to perform new studies with the same kind of NCS but adding solvent: what is potentially confined in the wax matrix should then be released in the medium and exert their adverse effects. We present and discuss these results on their ecotoxicity properties and the conclusion that we can draw from such results: can these paste-like complex mixtures exert a chemical adverse effect on aquatic organisms or is it unlikely?

TUPC19

Environmental and consumer exposure to sensitizing fragrances in household products - a case study

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Fragrances play an important role in household products. This is not only true for personal care products (PCP) with the primary intention of a "good scent" like perfume or deodorants, but also for all other kinds of PCP and also washing and cleaning agents. It is widely recognised that a lot of these fragrances have sensitizing potential and several of them are toxic for the aquatic environment. 26 fragrances which are well known allergens have to be named on the ingredients list of PCP and washing and cleaning agents (WCA). The goal of this study was to identify these 26 substances in household products (PCP and WCA) that do not have the primary purpose of providing a good scent. Members of 131 private households in Northern Germany were interviewed and all WCA and a selection of PCP that were used in the households were registered. The scanned PCP were limited to shampoo, body wash, bath additives, conditioner, soap, toothpaste, mouth wash, body lotion, hand cream, hair styling products, hair dye and makeup remover. Even though perfumes and deodorants were not scanned, the 26 selected fragrances were named more than 3300 times on the lists of ingredients (n=2711 products). Linalool and hexyl cinnamaldehyde were found most often. Linalool was present in almost 35% of the PCP to be left on skin or hair and hexyl cinnamaldehyde in almost 20% of these products. In general, sensitizing substances were most often found in "leave on"-PCP, almost 50% of these products contained one or more sensitizing substances, while only 37% of washing and cleaning agents contained such substances. WCA listed, on average, 1.1 sensitizing fragrances per product, "rinse off"-PCP listed an average of 1.5 sensitizing fragrances and "leave on"-PCP listed 1.7 sensitizing fragrances on average. The results show the high exposure of consumers to sensitizing fragrances, especially via "leave on"-PCP with a long exposure time. They also show that the exposure of the environment to these fragrances via "rinse off" products and WCA is significant, considering that products like washing powder are normally used in much larger volumes compared to "leave on"-PCP. The high number of persons with fragrance contact allergies and the presence of some fragrance compounds in the aquatic environment underline the importance of such studies and plead for improved regulations of these substances for an enhanced consumer and environmental protection.

Alternative approaches to animal testing for (eco)toxicity, and the regulatory application of the 3Rs in chemical risk assessments (PC)

TUPC20

Development and functional characterization of 3D spheroidal aggregate cultures of the rainbow trout liver cell line RTL-W1

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In vitro experimental systems based on piscine cell lines have been proven to be a powerful tool to unravel mechanisms of toxicity of chemical compounds. However, most continuous cell lines have the shortcoming that they have partially or completely lost tissue-specific functionalities resulting in uncertainties regarding their suitability to predict *in vivo* toxicity. It has been demonstrated that cell lines of mammalian origin can regain certain tissue-specific functionalities when they are grown as three-dimensional (3D) spheroidal aggregates cultures. Our objective is to develop and functionally characterize 3D spheroidal aggregate cultures based on the rainbow trout liver cell line RTL-W1 for environmental toxicity testing of single compounds and mixtures. The RTL-W1 cell line has been selected because of its wide use in environmental toxicology research. Furthermore, it has been demonstrated to exhibit several important liver-typic functions including cytochrome P450-dependent mono-oxygenase and ABC transporter activities. In addition, recent findings suggest that RTL-W1cells share morphological and functional characteristics with bile preductular epithelial cells, which function as stem cells in teleost liver. Moreover, it has been demonstrated that RTL-W1 cells grown in agar form aggregate cultures and show hepatocytic differentiation regaining structural and functional features of hepatocytes in their physiological environment. In our study it was observed that RTL-W1 cells form spheroidal aggregates cultures when cultured in suspension culture plates under continuous movement on an orbital shaker. Spheroid formation was initiated via collision of single cells resulting in loose cell clusters within the first 24 h. Cell density and rotational speed were identified as important parameters determining cell cluster formation and growth. Cell clusters increased in size in the following days by recruiting and binding more cells to their surface. After 4 days relatively compact spheroidal aggregates of uniform shape and size (~100 μm) had developed. At present the spheroids are being structurally and functionally characterized, e.g. regarding the viability of the cells in their interior, expression levels of hepatocyte-specific genes, and response to chemical epoxsure. Preliminary results suggest that RTL-W1 cells grown in three-dimensional spheroidal aggregate cultures are viable (positive alamarblue staining) and may have different properties compared to those grown in conventional monolayers.

TUPC21

Usefulness of environmental RAAF in metals industry - example of the PGM Industry

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The Platinum Group Metals (PGM) Industry needs to register more than 60 substances under EU REACH by the May 2018 deadline. The PGMs are typically low tonnage metals and metal compounds with a high monetary value and with typical uses as alloying elements, as catalysts or in electronics. An intelligent testing strategy (ITS) was developed for each relevant metal group (such as Platinum and Pt compounds, or Palladium and Pd compounds) by the PGM Industry in 2011 to meet the REACH registration requirements. The ITS considered the already available data vs. the REACH testing requirements, with the aim to minimize the need for vertebrate testing (cfr. 3Rs in chemical risk assessments). Care was taken to ensure a conservative conclusion for e.g. environmental threshold values and risk assessment. The major route applied in the ITS is the use of read-across within each PGM. In 2015, ECHA published the Read-Across Assessment Framework (RAAF), which sets the framework for reporting read-across under REACH. The RAAF was first published for human health endpoints. End 2016, the RAAF for environmental endpoints (fate and ecotoxicity) is at its final stages of development, and publication is expected soon. This presentation aims to verify the (non-)applicability of environmental RAAF for the PGM testing strategy, which was developed well ahead of the RAAF publication. The conclusions are expected to be representative for other non-ferrous metal REACH dossiers: metal specificities are common amongst the non-ferrous metals and might be factors (potentially) hampering the RAAF applicability.

TUPC22
A cross-sector review of global requirements for acute fish toxicity testing - opportunities for harmonisation and implementation of the 3Rs
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The acute fish toxicity test has been a cornerstone of ecotoxicology safety testing for more than 30 years. The key effect in this study is lethality, in order to generate an LC50 endpoint, and as such it has potential to cause significant suffering over the course of the 96 hour test. Considering these concerns, together with the advances in ecotoxicological methods since their initial inception, there are questions as to the intrinsic value of these tests and whether acute fish tests could be refined or replaced. In vivo fish acute tests are a core requirement under many chemical regulations globally (e.g. plant protection products, biocides, industrial chemicals). However, in some sectors and regions alternative approaches are accepted (e.g. cosmetics, national effluent testing) or this requirement has been fully removed (e.g. pharmaceuticals). Here an Ecotoxicology Working Group, initiated by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) and involving experts from across government, academia and industry, will present a review of the global fish acute test data requirements for all the major chemical sectors. This review will identify the drivers for the test (e.g. historical and scientific reasons such as the protection goals, and exposure scenarios under assessment) and highlight similarities and differences in the employed testing and assessment strategies. This analysis will highlight: (a) recommendations within and across sectors and regions, to streamline testing within current requirements; (b) highlight potential for cross-sector/regional harmonisation of requirements; and, (c) identify potential for harmonised implementation of alternative approaches (e.g. QSARs, fish embryo toxicity testing, threshold approach). This information aims to contribute to the development of short- and long- term strategies to replace, reduce or remove the core requirement for fish acute toxicity testing for the global assessment of substances or effluents, without compromising environmental protection.

TUPC23
Applicability of the in silico ecotoxicty prediction tool for pharmaceuticals in environment
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Chemicals used as pharmaceuticals are finally discharged into the aquatic environment as well as industrial chemicals. There are some concerns for environmental impacts of the pharmaceutical due to the unintended environmental effects, which may be different from biological medicinal effects. Recently, medical regulatory agencies require the pharmaceutical companies to submit the assessment reports of environmental impacts before the new drug products are marketed. It would be useful to predict the ecotoxicity of the new drug at the developmental stage, because the ecotoxicity studies are usually conducted at the final developmental stage just before submission. The ECOSAR software is well known as the *in silico* ecotoxcity prediction tool for environmental chemicals, and predominantly focusing on the industrial chemicals. We have evaluated the applicability of such kind of tool for prediction of ecotoxicity of pharmaceutical chemicals. We used the ecotoxicity test data sets of about 50 pharmaceuticals published by European Medical Agency. The NOEC values for the Daphnia magna reproduction and for the fish chronic toxicity were compared with the prediction NOEC values estimated by the ECOSAR. The percentages of the pharmaceuticals of which the predictive values are different in less than one digit from the actual measured values are 44% and 49% for the Daphnia magna reproduction and for the fish chronic toxicity, respectively. The predictive values trend to be higher than measured values in hydrophilic pharmaceuticals with lower octanol-water partition coefficient. While, the percentages of the pharmaceuticals of which the predictive values are different in more than two digits from the actual measured values are 13% and 30% for the Daphnia magna and for the fish toxicity, respectively. This difference suggests that the numbers of the pharmaceutical specific ecological toxicophore for the fish toxicity are more than those for Daphnia magna toxicity. In order to improve the predictability of the *in silico* ecotoxicty QSAR tool, more researches on discovering the pharmaceutical specific substructure in relation with ecotoxicity would be needed. This work was supported by the Research on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and Development, AMED.

TUPC24
A tiered testing strategy for rapid estimation of bioaccumulation by a combined modelling - in vitro testing approach: identification of candidate test chemicals
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Bioaccumulation is the net result of competing rates of chemical uptake and elimination in an organism, i.e., toxicokinetic (TK) processes, and is a key aspect of environmental hazard and risk assessment. Bioaccumulation can be assessed using metrics such as the bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF) and trophic magnification factor (TMF). The BCF is a common metric of regulatory interest determined from laboratory experiments in which the organism is only exposed to chemical in the water, i.e., OECD 305 TG. Laboratory guidelines for measuring BMFs from dietary exposures only have also been developed, i.e., OECD 305 TG. Field data and model studies for bioaccumulative chemicals show that the relative route of chemical exposure can shift from predominantly gill uptake to predominantly dietary uptake at log $K_{ow} \approx 5 - 5.5$. There is a need to reduce animal testing and develop alternative strategies for chemical evaluations. In silico models provide viable alternatives to vertebrate testing; however, predictive models require reliable parameterization data such as biotransformation rates which can significantly mitigate bioaccumulation. This project seeks to improve alternative methods to estimate bioaccumulation of organic chemicals in fish. We propose a tiered strategy that integrates TK models, quantitative structure-activity relationships (QSARs), in vitro experimental data from fish liver, gill, and intestinal tissues, and in vitro-to-in vivo extrapolation methods. To develop and evaluate the testing strategy a list of candidate chemicals for in vitro testing was developed based on: 1) model predicted discrepancies (hypotheses) between lower tiered one-compartment TK models and higher tiered multi-compartment TK models over a range of K_{OW} and biotransformation rates, 2) availability of reliable quality in vivo BCF and BMF data, and 3) availability of in vitro biotransformation rate data. These chemicals were further divided into three K_{ow} categories based on predominant exposure route(s) to guide hypothesis testing: 1) log K_{ow} ow 4 - 5.5 (mixed exposure routes); and 3) log K_{ow}>5.5 (predominantly dietary exposure dominates). This poster will describe the overall goals of the project and the chemical selection process.

TUPC25
Reducing the number of fish used in acute toxicity testing: Incorporation of the Fish Embryo Acute Toxicity test into the threshold approach
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The assessment of aquatic toxicity is required in various regulatory frameworks and has a huge impact on the number of fish used around the world. In 2011, nearly 180,000 fish were used for toxicological and other safety assessments in Europe. Large numbers of aquatic toxicity tests are required for REACH and, with the 2018 registration deadline fast approaching, strategies to reduce the number of aquatic

toxicity tests are urgently needed. The acute fish toxicity test (AFT, OECD Test Guideline [TG] 203) is one of the most frequently used aquatic toxicity tests worldwide. Because death is the endpoint, animal welfare is a significant concern, making the goal of reducing the number of animals used a priority. Applying the threshold approach (OECD Guidance Document 126), where an initial fish test is conducted at one concentration derived from test responses in *Daphnia* and algae and continued testing is triggered only if mortality is observed at this threshold concentration, can significantly reduce the number of fish used in the AFT. Furthermore, the Fish Embryo Acute Toxicity Test (FET, OECD TG 236) provides a significant refinement to the AFT, as embryos are used instead of adult/juvenile fish. We are developing a strategy for incorporating the FET into the threshold approach to provide a means for reducing the number of fish used in acute aquatic toxicity testing. This strategy is building on extensive earlier work and drawing upon the work of three individual efforts.Firstly, a new database containing acute toxicity data for adult/juvenile fish, fish embryos, *Daphnia* and algae has been constructed to analyse how the FET can be incorporated into the threshold approach. Secondly, the applicability domain of the FET is being clarified, and the uncertainties of the FET (focusing on metabolism) are being compared to the uncertainties of the AFT for the protection of the aquatic ecosystem. On this basis, a concept for defining acceptance criteria for the new approach will be proposed. Thirdly, a recently published report by the European Chemicals Agency on the use of the FET for REACH is being considered. This poster presents progress to date. *The views, conclusions and recommendations are those of the authors and do not necessarily represent the policies or positions of the organisations to which the authors are affiliated.*

Nanoparticles and Microplastics: Harvesting Recent Findings to Fertilize a New Pressing Topic (PC)

WEPC01

Adverse effects of particles: 2017 state-of-the-art of engineered nanoparticles as a platform for micro-nanoplastics research

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Donaldson et al. already in 2004 (Occup. Environ. Med., 61(9), 727-728) suggested that: "a new discipline should be built up to address the new potential threats posed by the widespread use of engineered nanoparticles (ENP)". Ever since, the nanotoxicology field has proliferated considerably, which is evidenced by an enormous number of publications and projects funded. A number of questions regarding ENP fate and effects have already successfully been resolved during the last years. In parallel to nanotox., also in 2004, Thompson et al. (Science 304(5672), 838-838) among first reported the occurrence of microscopic plastic fragments and fibers in oceans, hence termed micro-nanoplastics (MP). While a number of reports on the occurrence of MP in various habitats have been published already until 2010, the assessment of environmental toxicology of MP has begun only recently. The authors of this presentation have been involved in several EU FP7 and Horizon 2020 funded projects since 2010 and thus have access to the *state-of-the-art* knowledge in the field of nanotoxicology. For the purpose of this work a historical overview of the most important questions/assumptions that have been raised in nanotoxicity since its beginning were made: the importance of primary and secondary properties of ENP that govern the toxic effect, the contribution of ENP aging/transformation to toxicity, the importance of ENP characterisation, and the role of harmonisation and validation in toxicity testing. In parallel, existing knowledge regarding the properties of micro-nanoplastics (MP) was gathered. We present the state-of-the-art in ENP toxicity testing and provide guidelines for future MP testing. It is assumed that for toxicity studies most transfer of knowledge with regard to particle specific issues will be possible, as here extensive experience with a number of aquatic and terrestrial test systems exists. However, all specifics of the plastic material, and their implications for toxicity testing will require the generation of new knowledge by novel approaches. As the scope of this SETAC session describes, cross-fertilisation between these two fields of environmental toxicology is necessary to successfully implement the knowledge already gained through numerous projects and avoid duplication of incorrect assumptions.

WEPC02

Mapping the flows of 5 plastics through society: a first step towards building an environmental release model for plastics

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Evaluating the magnitudes of flows and concentrations of anthropogenic pollutants in the environment forms the basis of any environmental risk assessment. In the past years microplastics have received growing attention from the scientific community as particulate pollutants that could potentially pose a threat to human health and the environment. Current concentration measurements are more qualitative than quantitative, but for risk assessment it is very important to get information on environmental exposure and environmental concentrations in addition to hazard data. The goal of this project is to develop a material flow model that will describe the flows of different plastics to the environment, as macro- and microplastic, using data on plastic production and use and considering the life-cycle of products. The different plastics of interest for this project are the ones that are most reported in the environment: polyethylene (PE), polypropylene (PP), polystyrene (PS), polyethylene terephthalate (PET) and polyvinylchloride (PVC). In a first step a material flow model for the various plastics is developed that maps the flows of the five plastics through society: from production, to use and disposal. The model that is used is a dynamic probabilistic Material Flow Analysis method [1], as has already been used to model the engineered nanoparticles' releases to the environment [2]. The dynamic approach permits to model a varying production on a yearly basis, as well as the flows into and releases from stocks. The probabilistic approach using Monte-Carlo methods permits to model the uncertainty linked with estimates and the lack of data. While the here presented first module of the model allows to predict the amount of waste caused by items produced in the past years, once the second and third modules are finalized, the model will permit to identify the major sources of plastic to the environment. [1] N. A. Bornhöft, T. Y. Sun, L. M. Hilty, and B. Nowack, "A dynamic probabilistic material flow modeling method," Environ. Model. Softw., vol. 76, pp. 69–80, 2016.\n[2] T. Y. Sun, N. A. Bornhöft, K. Hungerbühler, and B. Nowack, "Dynamic Probabilistic Modeling of Environmental Emissions of Engineered Nanomaterials," Environ. Sci. Technol., vol. 50, no. 9, pp. 4701–4711, 2016. *Acknowledgement - The authors thank BAFU for funding this research project.*

WEPC03

A potential for knowledge transfer: parallels and differences in aquatic ecotoxicology between engineered nanomaterials and micro- and nanoplastics

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Research over the past few years has shown that microplastics (< 5 mm) are widespread in aquatic ecosystems worldwide. The continuous fragmentation of plastics suggests that also particles in the nanometre size (i.e. nanoplastics) are present in the environment. Due to their small size micro- and nanoplastics can be ingested by a wide range of aquatic animals, causing direct ecotoxicological effects of the particles themselves and vector effects, caused by associated co-contaminants. Additionally, adverse effects may result from monomers and plastic additives that are released from the polymer matrix. Even though knowledge on ecotoxicological effects of micro- and nanoplastics is still limited at present, their abundance and small size give rise to concern for impairments of organisms on different levels (from molecular to physiological) and translocation of particles inside organisms. These are issues that are also often discussed for engineered nanomaterials. Also the transport of co-contaminants and leaching of soluble substances are much debated issues in the field of nano-ecotoxicology. The similarities between the fields of engineered nanomaterials on the one side and micro- and nanoplastics on the other side call for a critical comparison and knowledge transfer. We therefore present an overview of similarities and differences between the two fields and conclude which lessons can be learned from the research on nanomaterials and transferred to the work with micro- and nanoplastics. The main focus is thereby on the particle properties and appropriate test design to analyse potential ecotoxicological effects of particles on aquatic organisms. We discuss the advantages and disadvantages of standardised tests as well as the aspects that need to be considered to achieve a good exposure control and characterisation. Identified key lessons include: the implementation of systematic particle characterisation, leaching controls, the use of reference material and setting clear definitions for different particle types. Even though many aspects are comparable it is equally important to understand where similarities end. Micro- and nanoplastics are likely to present different methodological and environmental problems, which need to be considered alongside the 'learned lessons' in the planning and interpretation of experiments.

WEPC04

Microplastic and nanoparticle fate in aquatic environments: learning from similarities and differences

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A growing concern related to the presence of microplastic particles (MPs) in marine and freshwater environments has unveiled significant uncertainties and open questions regarding their fate and exposure assessment. These fall mainly into the domains of (1) elucidating sources and emission pathways, (2) developing analytical tools for characterization and monitoring in complex matrices, (3) identifying relevant environmental transformation and transport processes and (4) designing adequate experiments to study these processes in the laboratory. These questions are strikingly similar to issues addressed in the field of research on potential environmental implications of engineered nanoparticles (ENPs) in the past decade. Considering the particulate nature of both engineered nanoparticles (ENPs) and MPs, it quickly becomes apparent that parallels can be drawn between the two groups of emerging contaminants and may serve to enable a more efficient development of the tools and methods required to support MP exposure and ultimately risk assessment. In this contribution we carefully evaluated the similarities and differences of ENPs and MPs with respect to their composition, intrinsic properties, applications and sources, potential release and emission pathways and relevant analytical target parameters. Our analysis revealed several commonalities between ENPs and MPs, mainly based on their particulate nature. Consequently, fundamental particle transport processes, described by colloid science, apply to both ENPs and MPs making modelling frameworks developed to predict ENPs fate easily adaptable to MPs. However, different typical densities and size ranges of ENPs and MPs need to be accounted for, as well as specific fate processes, such as leaching of additives upon aging of MPs. Overall, it became clear that taking advantage of existing knowledge on ENP fate will avoid re-inventing existing methods and help focus our efforts on the more pressing research needs, e.g. in areas where MP and ENP are rather different. This includes studying MP-specific fate processes, such as ageing and release of (potentially harmful) additives and the development of analytical methods to detect and characterize MPs in complex environmental matrices. Additionally, the historical development of ENP exposure assessment was examined and a need for improving interdisciplinary collaborations in the fields of emerging particulate contaminants was identified.

WEPC05

Electron microscopy methods for metallic nanoparticles applied to nanoplastics: Challenges and opportunities.

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Transmission electron microscopy (TEM) is a powerful technique to analyze accurately the size of nanoparticles (NPs) and is indispensable to characterize NPs in natural samples. Typically, sample preparation methods consisting in drying a small volume of NPs suspension on a substrate has a low number of NPs recovery (i.e. < 10%), form aggregates and are consequently not representative of the analyzed samples (Baalousha et al., 2014). Interestingly, new sample preparation methods for metallic NPs forcing a stronger adsorption on a functionalized surface substrate and using centrifugation have showed to be more efficient (i.e. recovery up to 71% and concentration correlation mass/number of NPs > 95 %) and result in evenly distributed particle on the sample carriers (Baalousha et al., 2014). The aim of our work is to develop a standardized sample preparation method for TEM analyses of nanoparticles (including nanoplastics) that will be used in environmental studies. Here, we present the challenges of applying a method first developed for metallic NPs to nanoplastics. For instance, nano-polymers presents several challenges, such as their low particle density, their shape and their surface charge, complicating their deposition on the substrate via centrifugation. By using different centrifugation methods, we are developing an approach that induces a good adsorption on the TEM substrate. This development will have a high environmental relevance as it will (i) allow to accurately measure the size distributions and concentrations of nanoplastics (ii) will contribute to work at environmental nanoplastic concentrations and thus (iii) will contribute to numerous studies that need these accurate measurements such as nanotoxicology studies that aim at assessing of the risk of nanoplastics in the environment. Baalousha M, Prasad A, Lead JR (2014). Environ Sci Process Impacts 16:1338–1347. doi: 10.1039/C3EM00712J

WEPC06

Uptake of differently sized polystyrene nanoparticles by D. magna and influence of protein corona as a function of presentation mode

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Nanoparticles (NPs) can be defined as having at least one external dimension between 1-100nm. Due to their small size, NPs have a large surface area to volume ratio giving them unique characteristics that differ from bulk material of the same chemical composition. As a result, these novel materials have found numerous applications in medical and industrial fields with the result that environmental exposure to NPs is increasingly likely. Increased reliance on plastic, which degrades extremely slowly in the environment, is resulting in increased accumulation of micro- and nano-plastics in fresh and marine waters, whose ecotoxicological impacts are, as-yet, poorly understood. NPs are known to adsorb macromolecules from their environment, to form a biomolecule corona, which ultimately changes the NP identity and how it interacts with organisms. Thus, the presentation mode of plastic (and other) NPs is an important point to be considered when designing uptake and toxicity studies, as altering factors such as presence or absence of food can influence the uptake and excretion of NPs by organisms such as *D. magna* as a result of different biological macromolecules being available to absorb to the NPs surface. Here, we assess the impact of presenting NPs to Daphnia magna via the water phase alone, via the water phase in the presence of algae as a food source, and via algae that were pre-incubated with the NPs which were subsequently isolated to remove any NPs free in solution. We report here on studies, including uptake and excretion of two different sized polystyrene NPs (50 and 500 nm) under various presentation modes such as bare exposure, feeding during excretion and being incubated with a food source algae *Cholera vulgaris*, and further investigate on how secretion of biomolecules by *Daphnia magna* impacts NP uptake, stability and toxicity. Interestingly, larger NPs are taken up more due to their closer size proximity to a natural food source, and regardless of NP size or presentation method, a minimal amount of NP remains within the gut over a short-term period bringing fourth the importance in assessing bioaccumulation.

WEPC07

Synthetic textiles as a source of microplastics from households

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The laundering of synthetic textiles made of polyester can produce microplastic (MP) fragments or fibers due to the mechanical stress of the washing process and subsequently be released into the wastewater treatment system with the wash water. Fibers similar to those observed in household sewage effluent have been observed downstream of water treatment plants and can have a long residence time in receiving fresh waters. Lessons learned from studying nanomaterial release from textiles have been applied here to study the release of fibers from synthetic textiles; namely using standardized reference materials and systematic laboratory experiments to suggest mechanistic reasons for fiber release. The aim of this research was to characterize and quantify released fibers in terms of size distribution and total mass under a series of different wash conditions, pre-treatments and textile characteristics. Triplicate samples of two synthetic textiles made of 100% fibers were knitted into two different weaves (interlock and jersey), washed under three different conditions (DI water, liquid and powder detergents) and washed five sequential times, with filtrates analysed after each wash cycle. Additional variations included a sunlight pre-treatment, increased mechanical stress and excess surfactant in the wash solution. Using microscopy and image analysis, all fibers were analysed on a filter (typically n > 3000) to measure size distribution and total mass. Fibers released from jersey fabric were typically overall smaller in length than those released from the interlock fabric but generally the size distribution remained steady through multiple wash cycles for both knit types. Detergent surfactant seemed to be a decisive factor in the quantity of fibers released, where powder detergents mobilized the highest number of fabrics from the textiles and washes with DI water shed the least. Due to the looser knit, interlock textiles released a higher mass of fibers than jersey textiles. Through systematic study of the textile properties (i.e. knit variants) and use patterns mimicking the life cycle (i.e. detergent variants, wash cycles) we are able to identify the most important variables for MP fiber release during textile laundering and this information may lead to manufacturers choosing a different textile weave when possible or recommending different laundering conditions to minimize the release of MP fibers from products.

The challenges of Life Cycle Sustainability Assessment (LCSA) of energy technologies (PC)

WEPC08

Life cycle comparative study of individual and collective systems for residential thermal energy production and distribution: a case-study from Tuscany - Italy

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EU targets in energy policy to be achieved by 2050 focus on both renewable energy and energy efficiency and the life cycle approach to evaluate the sustainability of related technologies can provide a holistic assessment to support innovative energy policies also at the local level.\nWithin the project Geo4P, focused on the feasibility of shallow geothermal energy (SGE) resource based systems in a local urban area in Pisa, Tuscany (IT), we implemented a LCA-based comparative study among individual and collective systems for the production and distribution of energy for heating, cooling and sanitary hot water in residential applications. We considered, as renewable energy sources (RES), shallow geothermal energy, biomass and solar thermal energy and district heating to serve the collective systems. \nGrounding on largely site-specific data, the results of the study suggest that:\n- compared with the benchmark scenario based on natural gas, RES always deliver a benefit on the climate change category, while there is a variability for other impact categories which, in some cases, are even more impacting; \n- compared to individual systems, collective systems show improved performances for solar and biomass scenarios, no significant difference for SGE and worse performances for the benchmark scenario for all the impact categories; \n- the sensitivity analysis to evaluate the effect of population density in collective systems, by modeling different sized buildings located on the same area, shows only a slight inversely proportional correlation for some impact categories. \nThe life cycle costing (LCC) analysis for individual scenarios includes the contribution of investment, operation and maintenance costs over a service life of 20 years. Compared to the standard scenario, solar and SGE have lower LCC value due to moderate operational costs and, to the lowest initial investment for solar while, for SGE, to a high initial investment partially compensated by its residual value at 20 years. On the contrary, biomass scenario has the highest LCC value due to the high annual maintenance and biomass fuel costs, especially if pellet biomass is used.\nThe results of this study show that life cycle thinking tools are useful to deal with multifaceted aspects of sustainability, providing a range of information useful already in the planning stage of a project and therefore may be a powerful tool for policy makers for a balanced planning of future energy infrastructures at local level.\n\n

WEPC09

Methodological implications on the greenhouse gas balance of 2nd generation biofuels - ISO vs. RED (2009/28/EC)

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Currently the LCA methodology is used in varying shapes for evaluating environmental impacts of biofuel production. In a European context especially the LCA methodology used in context of the Renewable Energy Directive (RED 2009/28/EC) has to be pointed out as it aims to establish a unified life cycle based calculation of greenhouse gas savings of biofuels compared to their fossil pendants. The RED calculation methodology is of high importance for the industry to get their biofuel products certified in line with European Union´s sustainability criteria. The RED life cycle based calculation method is opposite by ISO 14040 methodology, which allows more freedom of choice concerning allocation procedures and credits. Comprehensive literature review shows that there is a lack of studies dealing with these differences especially on their impacts on the overall greenhouse gas performance. In fact methodological choices have a great impact on the overall result. Therefore the aim of the current contribution is to examine the impacts of the freedom of choice in ISO 14040 LCA methodology compared to the RED life cycle calculation system demonstrated on the example of 2nd generation bioethanol production using cereal straw as feedstock. The bioethanol production from wheat straw with an ethanol yield of 0.326 MJ_{EtOH}/MJ feedstock input, an energy input of 0.509 MJ_{th}/MJ_{EtOH} and 0.076 $MJ_{e}l/MJ_{EtOH}$ is investigated. Process energy is generated via a CHP using natural gas. Lignin is assumed to be used for electricity generation. In the investigation according to RED (2009/28/EC) Annex V greenhouse gas emission assessment methodology by-product allocation according to the by-products energy content is applied. Using ISO 14040 Life Cycle Assessment (LCA) methodology the avoided burden approach is chosen to quantify greenhouse gas emission credits. The results clearly show that using the avoided burden approach – compliant with the ISO 14040 standard – leads to higher greenhouse gas savings of lignocellulosic ethanol compared to fossil gasoline. The saving compared to fossil gasoline ranges from 66 % based on RED (2009/28/EC) calculation methodology and 91 % under applying ISO 14040 compliant avoided burden approach. If renewable resources (e.g.: by-product lignin) is used for process energy generation a saving of approx. > 100 % can be achieved. Energetic process integration provides maximized savings.

WEPC10

Sustainability assessment of hybrid concentrated solar power/biomass mini power plant

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REELCOOP, an EU-FP7 funded project which stands for REnewable ELectricity COOPeration (www.reelcoop.com), aims to develop renewable electricity generation technologies and promote cooperation between several EU Partners. It addresses five important renewable energy areas: photovoltaics (PV), concentrated solar power (CSP), solar thermal (ST), bioenergy and grid integration, with developing and testing three novel prototype systems are representative of both micro-scale (distributed) and large-scale (centralised) approaches to electricity generation. A hybrid mini power plant with a nominal electrical output of 60 kW, that relies on a regenerative ORC as power generation system, have been designed and a prototype will be installed at National School of Engineering of Tunis (ENIT) (Tunis, Tunisia). In this paper the field test data from this prototype will be used to perform a Life Cycle Sustainability Assessment using a methodology based on MRIO extended with environmental, socioeconomic and social aspects. Results will be compared with other alternative systems (conventional systems).

WEPC11

Accounting for uncertainty in the assessment of material requirements and related climate change impacts of the energy transition

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The recent French "Energy transition act" has set stringent targets to increase the share of renewables in the French electricity mix. In this context, this study aims at quantifying the requirements for steel, aluminium, copper and concrete that would result from the French electricity transition by 2050. The consequences of these requirements in terms of climate change impacts are estimated. Given the significant uncertainties regarding material intensities of electricity generation systems, the study adopts a possibilistic approach to uncertainty representation and propagation. Three steps are distinguished in the calculations. The first step aims at setting the material intensity in terms of direct requirements for steel, aluminium, copper and concrete with respect to several electricity generation systems (in tonnes of materials / MW installed). In the second step, the cradle-to-gate climate change impacts of steel, copper, aluminium and concrete productions are calculated considering a consequential modelling approach. Finally, in the third step, the potential future capacities installed from 2012 to 2050 are drawn from the so-called « Decarbonization through electricity » scenario of energy transition in France by 2050, as defined by the French ANCRE. The results of material requirements and corresponding climate change impacts, as a consequence of the French electricity transition from 2012 to 2050, are expressed as a family of cumulative probability distributions for the proposals: *i)* "requirement for materials is lower than a certain value" and *ii)* "climate change impact induced by the production of these materials is lower than a certain value". This study additionnally computes a single indicator (so-called "confidence index"), as a weighted average of upper and lower bounds of the resulting family of probability distributions. In particular, considering this confidence index as the sole indicator of likelihood, there is a 20% risk that the production of steel, aluminium, copper and concrete, as a response to the French electricity transition from 2012 to 2050, induce more than 444 million tonnes of CO_2-eq. These results are intended to inform decision-makers in the most transparent way, for them to eventually decide whether the calculated risks (that material use and corresponding climate change impacts be larger than given values) are acceptable or not.

WEPC12

Comparison of aggregated site-specific flue gas emissions of global coal-fired power plants with a country-level life cycle inventory

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1. Motivation Current top-down life cycle inventories (LCIs) in the field of fossil electricity generation lack validation because aggregated bottom-up data for the same regional level of detail is not available in most cases. Furthermore, variability within each region is unclear and country-level data is insufficient for detailed regionalized life cycle assessment (LCA) based on recently published impact assessment methods. Thus a site-specific inventory for airborne coal-fired power plant emissions is prepared which is then aggregated and compared to the *ecoinvent v3.3* LCI database. With the results, conclusions for the future improvement of top-down as well as bottom-up life cycle inventories can be drawn. 2. Methods This work builds on unit-level technical power plant properties provided by the *PLATTS World Electric Power Plant (WEPP)* database. Additional data on these power plants is used as for example from the U.S. Energy Information Administration (EIA) *eGRID* database. Global coal input data is taken from the *US Geological Survey (USGS)* coal sample databases and combined with individual coal source information for the power plant units as well as trade data from the *exiobase* multi-regional input-output (MRIO) database. These are used to estimate airborne emissions per power plant unit and combined with actual emission data per power plant available from national Pollutant Release and Transfer Registers (PRTRs). Emissions are aggregated to country-level to be compared with *ecoinvent v3.3* coal power plant datasets. 3. Results and conclusions The results show good agreement between aggregated site-specific emission inventories and country-level inventories regions of the world. At the same time, the site-specific emissions give an indication where country-level data may be deficient and which dataset to prefer in case of contradictions between datasets covering similar geographies and technological levels. On top of the validation of country-level datasets, the site-specific emission data can be used for more in-depth analysis of emission patterns, regional life cycle impacts and pollution reduction policies.

WEPC13

Priorities of fossil power plant flue gas abatement in Europe

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1. Motivation Pollution from airborne emissions poses an immediate threat to human health in densely populated areas such as Europe. Thus regulation of those emissions is an important objective in such regions. The energy sector is known to be a major contributor to airborne pollution. Previous studies in the field of life cycle assessment (LCA) have focussed on general assessment of health impacts of emissions but have not considered the regional impacts because neither inventory data nor impact assessment methods have been available on such a high level of regional resolution. Instead, low and high population density have been distinguished as archetypes. This work aims at providing a clear regional picture of airborne emission impacts with a detailed regional life cycle inventory of fossil power plants in Europe on an individual unit level. It suggests ways for alleviating health issues with upgraded flue gas control for sulphur dioxide (SO2), nitrogen oxides (NOx) and particulate matter (PM). 2. Methods Power-plant-specific emission data from the European pollutant emission register *E-PRTR* is merged with a global power plant database containing data from sources such as the International Energy Agency's coal power atlas, EU power plant information, and the *PLATTS World electric power plant (WEPP)* database. Remaining gaps in the emission inventory are filled with *US Geological Survey (USGS)* coal data, estimates on coal consumption from published power plant capacity factors and a model for main pollutant emissions during fuel combustion. Potential emission reductions are then estimated by applying models for flue gas cleaning technologies such as flue gas desulphurization, particulate filters or selective catalytic reduction

for denitrification of flue gases. Impacts on human health and ecosystems before and after additional flue gas abatement are quantified by regionalized life cycle impact assessment (LCIA). Cost models are then used to estimate the corresponding expenses for installation and operation of these abatement technologies. 3. Results and conclusions The results of this work highlight the hot spots in European airborne emissions and show where modernization of fossil power plant flue gas treatment is most urgent. As such, they provide clear guidance for regulators and aid in driving the reduction of human health hazards from airborne pollutants in the energy sector.

Big data analysis of monitoring data: what questions can be addressed? (PC)

WEPC14

Re-organisation of a long-term monitoring network using moss as bio-monitor for atmospheric deposition by example of Germany

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The determination of atmospheric deposition can be accomplished using technical sampling devices (bulk samplers, wet only samplers), biomonitors or modelling. In Europe, since 1990 moss sampled every five years at up to 7300 places in up to 35 countries was used as biomonitor. In the moss specimens, heavy metals (HM), nitrogen (N, since 2005) and persistent organic pollutants (POPs, since 2010) were determined. Germany participated in all surveys with the exception of that in 2010. For the moss survey 2015, the biomonitoring network applied in the 2005 campaign should be reorganized. To this end, a complex statistically based methodology including a decision support system was developed and implemented. Its application yielded a network with a reduction of sample points from 726 to 402. By use of the data collected in 2005 the performance of the reorganized network did not reveal significant loss of statistical validity.

WEPC15

Spatial patterns and temporal trends in contamination of Norwegian coastal biota

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National-scale monitoring of contaminants in coastal waters of Norway has been carried out for more than 30 years, and has generated an extensive data set including concentrations of a wide range of contaminants (including extensive long-term data for metals and persistent organic pollutants, and for more recent years, data on emerging contaminants) in mussels, fish and sediments from a large number locations along the Norwegian coastline (from 57.4 to 73.8 degrees latitude). Using generalized additive modelling approaches we sought to: 1) identify spatial patterns and temporal trends in the contamination of Norwegian coastal biota (with a focus on blue mussels (*Mytilus edulis*) and Atlantic cod (*Gadus morhua*)); 2) assess temporal coherence across sites and regions; and 3) identify possible drivers of differences between sites and regions. Key drivers of spatial and temporal patterns that were identified include local contamination history (with several important contamination hotspots in historically polluted Norwegian fjords, as well as evidence of local recovery following reduction in point source emissions), as well as biometric parameters (including fish length and lipid content). Through detailed analysis of an extensive monitoring database, this study provides important insight into the distribution, temporal evolution and drivers of contaminant concentrations in coastal biota.

WEPC16

Understanding the response of zooplankton dynamics to multiple stressors in the North Sea

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Marine ecosystems are increasingly experiencing multiple disturbances as climatic change leads to changes in sea water temperature, salinity, and pH, while increased nutrient concentration and pollutants are important chemical stressors resulting from human activities. There is still a lack of quantitative data and understanding on how these chemical stressors and stressors resulting from climate change interactin marine ecosystems. In particular, it should be taken into account that physical stressors resulting from climate change can affect contaminant exposure and toxic effects and vice versa. Our study aims to get better insight in the relative contribution of various environmental drivers, including nutrients and pollutants to changes at the base of the pelagic food web in the Belgian Part of the North Sea (BPNS). Monthly sampling campaigns were conducted at twelve stations within the BPNS and the Belgian harbors from February 2015 to February 2016. Zooplankton samples were collected and environmental variables such as sea water temperature, salinity, pH, chlorophyll a concentration, nutrient concentrations and a selected set of priority pollutants were measured at each site. Calanoid copepods (Crustacea, Copepoda), being the most abundant group within the zooplankton were identified to species level, sex and developmental stage. Community analysis was conducted using Primer 6 & Permanova+. *Generalized additive modelling* (GAM) in R was used to determine the main drivers of change in the abundance and distribution of the dominant copepod species *Temora longicornis* and *Acartia clausi.* The zooplankton community of the BPNS was characterized, showing distinct seasonal and spatial trends over a one year period. *T. longicornis* and *A. clausi* show different dynamics in their spatial distributions and abundances and appear to be driven by different factors. By means of GAM we succeeded to explain a large proportion of the variability in both species. A GAM including temperature, chlorophyl a, SiO_4, $\sum$PAH, $\sum PCB_7$, salinity and N/P ratio is able to explain 94.1% of the variability in the densities of *T. longicornis*. For *A. clausi* temperature, chlorophyl a, SiO_4 and $\sum$PAH concentration explain 81.8% of the variability in densities. When optimized, these GAM will provide important tools to identify and quantify the relative contribution of multiple stressors on zooplankton species within the BPNS.

WEPC17

Data science to link chemical contamination with biological effects in surface water in Flanders, Belgium

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The Flemish Environment Agency is responsible for collecting monitoring data on surface water quality in Flanders (Belgium) in order to assess compliance with Flemish Environmental Quality Standards. The collected data include physico-chemical parameters, a broad set of specific pollutants (including metals, pesticides and PAKs), biological parameters (species counts/indices for macro-invertebrates, fish, macrophytes and phytobenthos) and river morphology parameters. This poster will demonstrate an improved understanding of the links between chemical contamination with biological effects. For this, multivariate statistical approaches (principal component analysis and linear discriminant analysis) on 10 year data set were used to link effects with contamination patterns in the matrices water, sediments and biota from freshwater systems. The main result indicate that contamination is inversely related to biological diversity and contamination tends to cluster around groups of emission types: urban pollution to water, agricultural pollution to water,...

WEPC18

Species Sensitivity Distributions for many substances used to provide a realistic estimate of ecological impact of mixture exposure in terms of the loss of aquatic biodiversity

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A conceptual framework of Ecological Key Factors (EKF) for the ecological assessment of water quality issues has been developed by the Dutch water quality authorities. The EKF describe conditions for good water quality. These factor descriptors help to structure the available information of water quality and make it possible to pinpoint dominant pressures on water system functioning, leading to effective restoration measures. Ecotoxicity has been identified as one of the EKF. In a series of five reports and two computer application tools the methodology to assess the EKF-toxicity is described along two distinct methodological pathways: 1) based on the evaluation of measured or modelled chemical concentrations using Species Sensitivity Distributions (SSD) and mixture toxicity evaluation, and 2) based on effect directed analysis using in vitro and in vivo bioassays. This presentation describes the chemical specific approach yielding a practical computer application capable of estimating the impact of a local mixture of chemicals on generalized biodiversity in terms of the predicted proportional loss of taxa. As a first step in the preparation of the EKF-toxicity tool, a detailed inventory was made of all substances (priority, listed, legacy, recent and emerging) that may pose a risk to European water bodies. This inventory yielded a categorized list of 6007 substances. From the substances in this list as many as possible toxicity data were retreived from a variety of sources. This exercise yielded a total of 94720 toxicity records, comprizing 2010 different substances, 2265 different taxa, 47367 acute EC50, 20280 acute NOEC, 2310 chronic EC50 and 24763 chronic NOEC values. Since we have the experience that the observed effects of toxicant exposure are not surprisingly more closely reflected by acute EC50 values than by chronic NOEC values, we extrapolated all data to acute EC50 values. Furthermore, the data richness and quality of the SSD dataset is categorized. The computer application to derive overall mixture toxic pressure expressed as the multi-substance Potentially Affected Fraction of species (msPAF) is programmed in MS Access. The application performs all calculations of toxic pressure in batch mode according to a mixed mixture evaluation model. Next to the overall toxic pressure, the application also identifies the top five toxicants with the highest contribution to the overall toxic pressure.

Environmental endocrine compound concentrations and human and ecosystem health effects (PC)

WEPC20

Effects on thyroid signaling and early neurogenesis of pesticides amitrole and chlorpyrifos

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According to epidemiological studies, the incidence of autism spectrum disorders has increased significantly over the past decades. Several studies demonstrate that the exposure to pesticides or biocides affecting thyroid hormone signaling might be responsible for this increase. In humans, embryonic exposure to pesticides during development of the nervous system increases the risk of autism by six times. Small model organisms allow the exposure of larvae during early embryonic development, which can be difficult to experimentally address in mammals. The Xenopus embryo is a unique and relevant experimental model that reflects the neurogenesis in mammals developing *in utero* and concomitantly offers an alternative to conventional *in vivo* tests. We examined the effects of two biocides that are under re-evaluation by the European Commission: chlorpyrifos and amitrole. Both xenobiotics are found in surface waters at ng/L concentrations and it has been recently shown that both compounds can be found in human fluids. Although only high doses have been shown to be toxic, the activity of amitrole on the thyroid gland has been demonstrated in several species. Our goal was to use transgenic THbZIP-gfp *Xenopus laevis* tadpoles to investigate the potential of amitrole and chlorpyrifos to disrupt thyroid signaling. We studied the effects of early embryonic exposure to amitrole and chlorpyrifos on the swimming behavior and longterm brain development. We also tested the hypothesis that embryonic exposure to amitrole and chlorpyrifos induces changes in the expression of the target genes of thyroid hormones and / or candidate genes associated with autism using qPCR. Both pesticides exhibited pro-thyroidogenic activity at concentrations 10^{-6}M (amitrole) and 10^{-10}M (chlorpyrifos). In addition, 10^{-12}M amitrole exhibited anti-thyroidogenic activity in the presence of T3 (5nM). Amitrol (10^{-8}M) and chlorpyrifos (10^{-10}M) significantly reduced the mobility of tadpoles. Both compounds significantly reduced the expression of mature neuronal marker *tubb2b* at the concentration 10^{-8}M. Chlorpyrifos (10^{-8}M) also reduced the expression of *cntn4* (a key gene in synaptic development). Our results suggest that amitrole and chlorpyrifos are exhibiting thyroid disruption activity. We are currently studying gene expression of autism related targets and long term effect of an embryonic exposure to amitrole and chlorpyrifos on brain structure.

WEPC21

Urinary concentrations of major phthalate metabolites in pregnant mothers and newborns in relation to thyroid hormones

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Fetus and infant are highly susceptible populations to endocrine disrupting chemicals. However, information on their exposure profile and associate risks during the early stages are very limited. This study was conducted to understand the exposure levels of major phthalates among pregnant women and newborns of Korea, and to determine their association with endocrine system including thyroid hormone balance. A total of 224 urine samples were collected from pregnant women at delivery. From 152 newborns, first urine samples were collected. Six phthalate metabolites for three phthalates, i.e., di(2-ethylhexyl) phthalate (DEHP), di-isobutyl phthalate (DiBP), di-n-butyl phthalate (DnBP), and diethyl phthalate (DEP), were analyzed in urine samples. Thyroid hormones (free T3/T4, total T3/T4, and TSH) were also measured in maternal and cord serum at delivery. Socio-economic status, and medical records were gathered from all participants. The metabolites of DEHP and DnBP were detected in 100% of the urine samples. Most of the metabolites were significantly correlated between maternal and newborn's urines. In maternal population, TSH concentration was negatively associated with MEP level. In newborn babies, urinary MEHP and MEP concentration was negatively associated with cord free T3 levels. MiBP and MnBP levels were also negatively associated with cord free T4. Maternal MEOHP concentration in urine was associated with increased odds ratio for low free T4 or total T4 in cord serum. Our observations show that phthalates exposure may be associated with the alteration of thyroid hormone balance in mother and newborns. Implication of thyroid hormonal change to fetus are of concern, and thus deserves further consideration.

WEPC22

Mixtures affecting neurodevelopment disrupt thyroid hormone signaling in Xenopus laevis.

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Endocrine disrupting chemicals (EDCs) harm human health both as single molecules and as mixtures. New strategies developed within the framework of the European EDC-MixRisk project is to start from human cohorts (SELMA and Life Child) where neurodevelopment of children (up to the age of 7) is followed up. Retrospective analysis of fluids (blood and urine of mothers) collected during pregnancy (7 years ago), allowed identification of a chemical mixture (mix N) associated with neurodevelopmental delay in these children.The main adverse health outcomes linked with this mixture are effects on cognition, behavior and motor development. Mix N is composed of phthalate metabolites, BPA and perfluorinated compounds. This mixture is being tested by a battery of in vivo and in vitro assays to investigate its potential disrupting effect on neurodevelopment. \nThyroid hormones (THs) are crucial for brain development. We hypothetized that this axis could be a key target for disruption and hence alteration of normal brain development. We have developed a Xenopus Embryonic Thyroid Assay (XETA) and used it to screen thyroid disruptors either with a spiked or non spiked protocol (with or without TH). Transgenic tadpoles, harboring a TH/bZIP-EGFP construct, were exposed for 72h to equivalent concentrations of 0.01x to 1000x human levels. Single exposure of the mix exhibited anti-thyroidogenic activity at 10x and 1000x concentrations. In co-treatment with T3 (5nM), Mix N exhibited anti-thyroidogenic activity at 100x and 1000x human exposure levels. An excitatory effect was induced by Mix N at 0.1x in the presence of T3. These results show that Mix N can disrupt thyroid hormone signaling in a non-monotonic manner. Gene expression measured in brains of tadpoles exposed to different concentrations of Mix N over 72h was analysed by qPCR. Our data confirm anti-thyroidogenic effect of Mix N 1000x on the transcription of *thbzip* and transcription factor *klf9* in presence of T3. Without T3, exposure to Mix N 10x downregulated expression of oatp1c1, a TH-transporter. These results show altered expression of genes involved in TH signalling pathway after exposure to different concentrations of Mix N. These data suggest that this new strategy of experimentally validating pre-identified mixtures of chemicals associated with adverse outcomes in human is promising. It offers a connection between adverse effects in human, chemicals and mechanistic data promoting better mixture risk management.

WEPC23

Urinary concentrations of organophosphate and pyrethroid metabolites from two rural Spanish populations

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Organophosphate (OP) and pyrethroid (PYR) pesticides are commonly used in agriculture and in domestic and gardening applications. Their metabolic action eliminates insects by disruption of their brain and nervous system. This neurotoxic effect is not selective enough to avoid deleterious inputs to other non-target species, including humans. Once in the human body, OP and PYR pesticides are metabolised and excreted in urine within 4-48 hours after exposure, depending on the compound. A new analytical methodology for the simultaneous quantification of OP and PYR urinary metabolites has been developed taking into account the wide range of concentrations of these compounds in humans from general and highly exposed populations, e.g. rural or agricultural areas. Six biomarkers of OP pesticides and three biomarkers of PYR compounds have been quantified by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). This methodology has been externally checked out by participation in rounds of the German External Quality Assessment (G-equas). A total of 125 urine samples from two adult Spanish populations have been analysed: the rural population of Sucs, in the West part of Catalonia (n=88), comprising both farmworkers (n=48) and individuals from the general population (n=40); and two populations from Galicia (n=37): one from the urban area of Santiago de Compostela (n=21) and the other from a rural township in Carbia (n=16). Concerning OP pesticides, TCPY (metabolite of chlorpyrifos) was the most abundant metabolite in all the samples analysed, with a median concentration of 18.8 ng/ml, followed by DEAMPY (metabolite of pirimiphos, found in 86% of the samples) and PNP (metabolite of parathion and methyl parathion, found in 99% of the samples), with median concentrations of 3.3 ng/ml and 1.9 ng/ml, respectively. The other OP biomarkers (e.g. IMPY, CMHC and MDA) were detected in few samples (< 6%). Among PYR pesticides, 3-PBA (metabolite of several commercial pyrethroids) was found in 91% of the analysed samples, with a median concentration of 1.7 ng/ml, followed by 4-F-3-PBA and transDCCA, which were found in 45% and 20% of the samples, respectively. For the major compounds (e.g. DEAMPY, PNP, TCPY and 3-PBA), the highest concentrations were found among farmworkers, followed by individuals from rural areas. The lowest concentrations were found within the individuals living in an urban area.

WEPC24

Prolonged test scenarios are required for assessing the effects of endocrine disrupters

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It is generally known that exogenous compounds that alter the function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations, so called endocrine disrupters (ED) according to the WHO/IPCS definition, are difficult to assess. Biological responses to ED induced during exposure in a sensitive stage during the life cycle of for example fish, might become observable in a later stage of development, during reproduction, or even in the next generation. Moreover, tresholds are difficult to establish since ED are often potent at very low (undetectable) concentrations and it was frequently observed that responses show non-monotonic relationships to the exposure concentration. Interference of ED with the natural feedback mechanisms of the hypothalamic-pituitary-gonadal/thyroid-liver axis depends on both external and internal additional stimuli, making reproducible endocrine testing extremely hard. We exposed male zebrafish of the same size and age individually to radiolabelled ethinylestradiol (^{14}C-EE2) for a short time under exactly the same conditions via two different routes (via the water: 1 μg ^{14}C-EE2/L and via the diet: 0.27 μg ^{14}C-EE2-equivalent in worms/fish). Uptake and elimination data (internal concentration of radioactivity) showed large variability between replicates (coefficients of variation up to 55%). Even larger scatter in the data was observed after calculating the ratio between the concentration of radioactivity in different fish compartments and in the water (coefficients of variation up to 70%). Hence, it was demonstrated that accumulation, distribution, metabolism, and elimination (ADME) kinetics may largely vary along individual animals of the same sex, size, and age, which are exposed for a short time (e.g. the interval between two water renewals in semi-static tests). Taking this together with the scientific discussion on delayed effects and non-monotonic dose-response relationships of ED, it seems that prolonged test scenarios (with a flow through design), as for example the Medaka Extended One Generation Reproduction Test (MEOGRT, OECD 240) and the Larval Amphibian Growth and Development Assay (LAGDA, OECD 241), are required to adequately assess an ED.

WEPC25

Chronic Arsenic Exposure Impairs Pancreatic β-cell Function

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Several natural and man-made environmental factors directly affect endocrine function. Clinical cross-sectional studies have identified positive correlations between exposure to one such factor, inorganic arsenic, and the prevalence or severity of type 2 diabetes mellitus (T2DM). This link presents a world-wide health concern, as over 100 million people worldwide are estimated to ingest unsafe levels of arsenic daily. Though clinical data are mixed regarding the severity of arsenic's effects on diabetes, animal and cell-culture models suggest that arsenic exposure decreases peripheral insulin sensitivity and β-cell function. Our laboratory observed that mice provided drinking water containing 50 ppm trivalent arsenic for 8 weeks exhibited glucose intolerance despite reduced HOMA-IR. Arsenic did not significantly affect peripheral insulin resistance, however, during an intraperitoneal glucose tolerance test, plasma glucose was significantly elevated at all time points post-injection. During this test, insulin levels remained similar to those of control mice, suggesting that the β-cell response to hyperglycemia was impaired. We interrogated this possibility using a model of chronic low-dose arsenic exposure in the MIN6-K8 pancreatic β-cell line. MIN6-K8 cells exposed to trivalent arsenic for 3 days in culture medium exhibited a robust, dose-dependent decrease in glucose-stimulated insulin secretion. Unexpectedly, glucose-stimulated calcium mobilization was significantly enhanced by arsenic treatment. These *in vivo* and *in vitro* studies provide evidence that pancreatic β-cell dysfunction plays a significant role in the link between arsenic exposure and T2DM, and comprise a robust model system by which we can interrogate the underlying mechanisms of arsenic-induced metabolic dysfunction.

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