Environmental Quality Through Transdisciplinary Collaboration
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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 Fleming1, N. McDonough2, M. White1, O. McMeel3, C. Domenech1, S. Wajts1, A. Blaauw1, J. Vera-Prieto4, M. Parga5, J.B Calewaert6, R. Gardside7, M. Garza8, M. Depleudge9

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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 academia, regulators and industry to work together to solve knowledge gaps and develop improved methods and approaches. One area to explore is to develop more integrated approaches between academia, regulators and industry to solve knowledge gaps and develop improved methods and approaches.

Furthermore, 'new approaches' could be integrated with existing approaches: ‘omics’, systems biology, bioinformatics, standardised ecotoxicology 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) M. Pauwels, University of Lille / UMR CNRS 6198 EVO-ECO-PALEO; M. Karbe, University of Lille / UMR CNRS EVO-ECO-PALEO; D. SOULEMAN, University of Lille1 / GGCGE Fonctionnement des Ecosystèmes Terrestres Anthropisés; H. Ferot, University of Lille / UMR CNRS EVO-ECO-PALEO 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 only 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 reproduce in metal-polluted soils). Arabidopsis halleri, in particular, occurs in non-polluted areas but also colonized anthropogenic calamine (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 non-tolerant 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 MTPI (METAL TOLERANT PROTEIN 1) encoding a Zn transporter involved in vacuolar sequestration. We therefore investigated the molecular evolution of MTPI from a large set of A. halleri natural populations. Resulting sequences (at least at generation 5) 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 killfish (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. Winfeld, 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 798 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 and epigenetic epiphenomena following a chronic external gamma irradiation in Daphnia magna M. Trijus, IRSN/PRP-ENV/LECO / PRP/ENV/SERIS/LECO; J. Assellent, Ghent University / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit: O. Armant, C. Adam-Guillemier, IRSN/PRP/ENV/LECO / PRP/ENV/SERIS/LECO, K. De Schampheleire, Ghent University / UGent / Applied Ecology and Environmental Biology; F. Alonzo, IRSN Apohl, 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 focused on the genetic impact of ionizing radiations 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 lifelong 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 steadily 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, miscoded 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 through epigenetic modifications. 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. Lancej, Central Queensland University / School of Medical and Applied Sciences; T. Cresswell, ANSTO Environmental Research / Institute for Environment and Security; A. Dohmen, BASF SE / Landw Versuchsstation APDRO Advances on the assessment of environmental pollutants to amphibians and reptiles
Through reciprocal crosses between a reference and contaminated population, and teratogenesis. We confirmed biparental inheritance and a genetic basis of protection. Chemical drivers of the selective sweeps that we see in populations from Galveston Bay, Texas, USA, have been found to contain substantial levels of mercury, dioxins, furans. The Houston Ship Channel (HSC) in Texas includes areas that have historically been colonized by anthropogenic calamine (zinc (Zn) and cadmium (Cd)) with traits, including metal tolerance (ability to survive and reproduce on Zn-polluted habitats). In parallel, an independent cross of the common toad (Bufo bufo) and common frog (Rana temporaria) in agricultural landscapes in Germany - results of a monitoring study. 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 management actions to improve species richness in agricultural landscapes for assessing potential impacts of agricultural measures.

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

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

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 watersheds 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 hydro-chemical water characteristics were measured, together with organic (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 conservation status is characterised by more sensitive taxa (such as Plecoptera, Ephemeroptera, Tricoptera), while resistant taxa (e.g. Chironomidae, Olgiacochaeta, 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 sediment used compared to the summer campaign, with more than 100 compounds being characterized (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. Diamanti, Tetra Tech, Inc.; A. Rosebery-Lincoln, C. Boschien, Tetra Tech; R. Kolly, 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 assemblies. 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 Ecology; K. Foit, UFZ - Helmholtz Centre for Environmental Research; S. Knillmann, Helmholtz-Centre for Environmental Research (UFZ) / Department of System Ecology; S. Schöberl, University of Freiburg 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 fail 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 toxipants (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 through a 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
B. Grussen, University of York / Environment Department; E. Butler, A. Frances, Unilever / Safety and Environmental Assurance Centre SEAC; S. Marshall, Unilever / SEAC; O. Price, C. Rendal, Unilever / Safety and Environmental Assurance Centre SEAC; R. Ashauer, University of York / Environment
The lack of ecological realism in current prospective environmental risk assessment (ERA) models is a limitation as compared to investigating processes that are driven by a multistressor 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 which is further influenced by 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 characteristics. The characteristic 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 biophysical fluxes. We can then use these stressors to be applied to the individual 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.

15 Fate and effect of sediment-associated triclosan: Impact of T. tubifex, sediment characteristics, and temperature
R. Wendland, Roskilde University / ENSPAC; D.T. Salvido, Research Institute for Fragments, Materials & Environmental Science; H. Selck, Roskilde University / Dept Science and Environment
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 examines 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 temperate 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 negligible, whereas uptake from sediment (i.e. grain size). Sediment characteristics were controlled by, for instance, the attachment 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.

16 In situ measurement of nanoparticles
C. Stephan, PerkinElmer, Inc.; R. Merrifield, University of Birmingham / geography earth and environmental sciences; J. LEAD, Arnold School of Public Health University of South Carolina / Center for Environmental Exposure and Risk (CENTER)
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 significant risk to 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 a 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 deparation of both dissolved (Au and Ag) (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 high-concentration exposures along with evidence for intercellular interactions with the NPs.

17 Predictability of silver nanoparticle speciation and toxicity in ecotoxicological models

J. Koeke, M. Engelke, University of Bremen / Center for Environmental Research and Sustainable Technology; M. Hoppe, Federal Institute for Geosciences and Natural Resources; A. Nogowski, Technical University of Dresden; J. Fishér, University of Bremen / Center of Environmental Research and Sustainable Technology; J. Tömming, University of Bremen / Center for Environmental Research and Sustainable Technology.

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 ultratricriffugation 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 the 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.

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

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Fullerenes are carbon-based nanomaterial composed by sp²-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 impact or anthroocenic sources, vehicle exhaust and brakes, some personal-care products, etc.). Recent studies have detected fullerene 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 transformation products C₆₀ 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 (functionalization of fullerene 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 determination 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 C₆₀ 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 times of simulated sunlight exposure and the kinetic of degradation were studied. Overall C₆₀ was observed to be a rather persistent compound, although different degradation products (mainly epoxides and dimers) were identified and semi-quantitised. The pH, salinity and humic substance content had a significant impact in the degradation of this fulleren and the presence of open cage compounds showed good degradation trends. This work was supported by the Spanish Ministry of Economy and Competitiveness through the projects INTEGRACOST (CGL-2014-56530-C4-1-R) and NANO-transfer (ERA_NET SINN PCIN-2013-182-C02-01) and UK National Environmental Research Council through the project Trojan Horses (NE/L006782/1).

19 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 flow analysis (PMFA) to assess the flows of nano-TiO₂, nano-ZnO, nano-CuO, nano-Ag, nano-SnO₂ 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 is 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.

20 Modelling nanoparticle fate in wastewater treatment plants

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Engineered nanoparticles (ENP) have been the subject of hazard investigations in the last 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 80 nm PEG coated silver ENP (Ag NP) to WWTP biosolids and their subsequent sedimentation simulating a clarifier was investigated, using biosolids form a WWTP in Gothenburg, Sweden and varying physical parameters as well as shear, and total suspended solids concentration (TSS). The goal was to provide insights in ENP and WWTP specific parameters explaining the variation in ENP removal in WWTPs. Larger Ag NP were found to attach faster to WWTP biosolids compared to larger ones, coating having no effect. Increasing shear rate had little effect. The combination of the 28 nm Ag NP coating with low shear was investigated. This work was motivated by sedimentation experiments suggesting that relatively smaller ENPs are more likely to leave the WWTP with the effluent. Physical parameters 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 ENP during transport through WWTP.

Metals in the Environment: Fate, Speciation and Bioavailability in Water, Soil and Sediment
Opposing gradients for undesirable and essential elements in saithe from 216.153.025-1.0 of the Research Division of the Universidad de Concepción. Pollution Control and Areas contaminated by Point Sources in Turkey. Concentrations of Cd, Hg, Pb, V, Cr, Mn, Co, Ni, Cu, Zn, As in urban areas were determined by several in vitro gastro(intestinal) methods including the SBRC, RBA As for use by contaminated site managers. 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.


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 Skaggerak, 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 for copper and zinc.

23 Assessment of Exposure to Heavy Metals via ingestion, inhalation and dermal contact to Urban and Background Soils in Turkey K. Akgöz, Bursa Technical University / Department of Environmental Engineering; P.B. Kurt Karakus, Bursa Technical University / Environmental Engineering Department

Heavy metals in aquatic soil and posive negative effects on living organisms and human. In this study, soil samples from urban and background sites located in Aksaray, Usak, Konya, Elazığ, Malatya, Kayseri, Mersin, Kırıkçukale, Kars, Kastamonu, Çankırı, İzmir, İstanbul, Antalya 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, 12315, 34035, 5649 µg/kg, respectively whereas it was 255, 9261, 18363, 40202, 159140, 5444, 577, 6662, 18023, 3821 µg/kg, respectively in background sites. It was determined that only the concentrations of As exceeded the Turkish Legislation on Soil Pollution Control and Areas contaminated by Point Sources in Turkey.

24 Predicting Arsenic Bioavailability in Contaminated Soils by Using In Vitro Gastrointestinal Bioaccessibility and Speciation for Site-Specific Risk Assessment N.T. Basta, The Ohio State University / School of Environment and Natural Resources; S. Whitacre, The Ohio State University; B. Stevens, US Army Corps of Engineers Engineer Research and Development Center; K. Scheckel, US EPA; H. Anderson, US Air Force; ORD/NCEA; V. Hanley, California Department of Toxic Substances Control

Exposure based on soil contaminated with As is assessed by human health risk assessment (HHRA). Extensive research efforts have been directed towards 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 IVIC regression equation is unknown. The objective of the current study is to evaluate the ability of several international bioaccessibility methods to predict As bioavailability in contaminated soils. 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 gastrointestinal (intestinal) methods including the SBRC, RBALP, USEPA Method 9200, OSU IV, and the California Bioaccessibility method (CAB). Relative bioavailable (RBA As) was determined from dosing trials using juvenile swine. Regression equations from published IVIC 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 IV ≥ RBALP, SBRC, USEPA 9200. The most accurate predicted RBA As values fell 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 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.

25 Assessing the human bioaccessibility of automotive exhaust catalysts in urban road dust J. Olsson, Uppsala Vatten; A. Horneman, University of Toronto / School of the Environment; J. Niu, Health Canada / Exposure and Biomonitoring Division; C. Levesque, M. Chénier, P. Rasmussen, Health Canada

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, 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 Palladium (Pd), Rhodium (Rh) and platinum group elements (PGE) in urban road dust using NS 5 assay-IAA. The lung simulated, 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 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(2.5x) > Rh(2.6x) > Pt(2.8x) > Ir(7). The observed enrichment of PGE in urban road dust is of considerable interest 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 bioavailability and toxic potential of PGE.

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

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

J. Ross, J. Burdick, A. Horneman, E. Houtz, H. Slenders, T. Pancras, ARCADIS 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 sulfonates (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 sulfonates and carboxylates. PFAS contaminated source zones are often associated with large plumes as the majority of PFAS are transported 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 as PFOS and 0.1 µg/L for PFOA 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.

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

S. Valasecci, M. Mazzoni, Water Research Institute - Italian National Research Council IRSA-CNR; M. Rusconi, Water Research Institute - Italian National Research Council / Water Research Institute; S. Polsetello, Water Research Institute- CNR / Water Research Institute; T. Ternes, Federal Institute of Hydrology; K. Jowell, German Federal Institute of Hydrology; BfG; B. Teiser, Abwasserverbund Braunschweig; J. Drewes, Technical University of Munich, TUM / Chair of Urban Water Systems Engineering; U. Hübner, J. Müller, Technical University of Munich, TUM The identification and the quantification of PFAS 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 toxic amount of PFAS precursors in natural water bodies, a method able to identify the precursors (and that can be formed by oxidation) for the quantification of the total amount of PFAS precursors in natural waters, after a persulfate 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 presence of PFAS precursors through the soil-surface waters 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 PFAS concentrations has been followed during all treatments steps, including the presence of potential precursors which can generate PFAS after end-of-pipe oxidative treatment before the distribution as potable water. The results showed that the soil infiltration test is able to generate PFAS from precursors already present in influent waters. The method for the estimation of PFAS 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 high has been always underestimated because industrial processes produces a significant amount of precursors that can be in vivo transformed in PFASs contributing to their environmental occurrence and bioaccumulation in the organisms.

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

L. Ahrens, Swedish University of Agricultural Sciences / Dept. of Aquatic Sciences and Assessment; L. Glimstedt, Swedish University of Agricultural Sciences; K. Wiberg, Swedish University of Agricultural Sciences / Dept. of Aquatic Sciences and Assessment; C. Cato, H. Holmström, Uppsala Vatten och Avfall AB; P. McLeaf, Uppsala Vatten; J. Olsson, Uppsala Vatten och Avfall AB 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 (2PFS = 55.000 ng L⁻¹ = 110.000 mg d⁻¹) with elevated discharges of C₃–C₅ perfluorocarboxylates (PFCCs). The studied WWTP was found to be ineffective in removing C₃–C₅ perfluorocarboxylates (PFCCs), C₆–C₇ PFASs and 6:2 FTSA from wastewater. Longer chained PFCCs (C₈–C₁₀) tended to partition to sludge more effectively than shorter chained PFCCs (C₃–C₅). PFASs and PFCCs tend to be at similar concentrations in the effluent compared to the influent indicating insufficient removal of PFASs within the WWTP.

29 Transport and Profiles of PFASs in the Arctic Ocean

L.W. Yeung, University of Oureibo / Department of Chemistry; S. Mabury, University of Toronto; R. Lohmann, University of Rhode Island / Graduate School of Oceanography 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 PFCCs; C₆, 8, 10 PFASs; McFOSAA and EfFOSAA, 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 Arctic layers which are persistent in the environment as they are not susceptible to further biodegradation 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 PFCCs, C₄, C₆, and C₈ PFASs. In the Atlantic water layer, only PFOS and PFNA were detected. Location North Barents Sea (PS80/227) is the only location where 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 farther from the Atlantic Ocean, such as Nansen Basin - West Gaekkel Ridge (PS80/254), Amundsen Basin - East Gaekkel Ridge (PS80/275), and near the North Pole (PS80/364), no detectable PFASs were observed in the Atlantic layer. The observed vertical profiles of PFASs in the current investigation may be explained partly by the circulation and water sources of the Arctic. There is little evidence that ocean currents transport PFASs into the deeper Arctic Ocean layers, which remain mostly PFAS-free.

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

H. Ronit, J. Aars, M. Andersen, The Norwegian Polar Institute; S. Bourgeois, University of Tromsø / University of Arctic Marine Biology, Arctic University of Bergen / Department of Biology; B. Jenssen, Norwegian University of Science and Technology / Biology; R. Lille-Langøy, University of Bergen; A. Polder, Norwegian University of Life Sciences / Department of Food Safety and Infection Biology; S. Kordbacher, Norwegian Polar Institute / Ecotoxicology; V. Torget, NTNU; J. Welker, University of Alaska Anchorage; Y. Yoo, UiT The Arctic University of Norway Perfluoroalkyl substances (PFASs) 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, focusing on the period 1992-2013.

We also analyzed carbon and nitrogen stable isotope ratios (δ¹³C and δ¹⁵N) in red blood
Wastewater effluents: How research can improve risk assessment and regulation

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


The conservation of Europe’s water resources was one of the main drivers, when CONCAWE (which stands for CONservation of Clean Air and Water in Europe) 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.

32 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


Active pharmaceutical ingredients (APIs) have been classified as emerging contaminants and, and their introduction into 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.

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

E. Uusitalo, Stockholm University / Baltic Sea Centre

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 conventional WWTPs was ca 50-60%. The weighted average RE based on typical industrial 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 equivalents would likely reduce or transform around 40-60% of the total chemical load from 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 is needed to enable better estimations of the total benefit of advanced treatment of wastewater.

34 Occurrence, characterisation and fate of (nano)particle 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 into 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 in nano/particle, 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 µg L-1) were significantly higher than Ag (<0.15–2.2 µg L-1). Most of the influent Ti and Ag occurred in particle form or was associated with suspended solids (>0.7 µm), while 5% Ag and 1% Ti was found to occur in colloidal or ion form. Different diurnal concentration profiles were observed for the two elements. Ti profile was found to follow PFRP 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 (which in most Ti carrying systems is difficult to occur in 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.

35 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 possibility to study in real environments consequences of a reduction in MP concentrations. Therefore, the goal of the present study was to establish trends in periphyton communities extracted from passive samplers from WWTPs effluents, periphyton which 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 the results were compared with a reference site (Eawag site) using LCIA methods that were deployed at the effluent of each WWTP. Bacterial and algal tolerance to the extracts was determined by measuring the inhibition of 3H-carbonate and 3H-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 concur with the tolerance measurements at this site, since no difference between up- and downstream periphyton was observed, unlike the site 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.

36 Life Cycle Data and Modeling Developments: challenges and solutions
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Uncertainty due to data limitations 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 exploration and exposure 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 3H-carbonate and 3H-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 concur with the tolerance measurements at this site, since no difference between up- and downstream periphyton was observed, unlike the site 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.

37 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 LCIs information captured by the LCIA methods. It allows an objective appraisal of LCIA by additional information on the adequacy and the completeness representation of LCIs 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 technique where the resulting dimensions have an environmental meaning. To compare 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 LCIA method, represented by the RI, is not determined by the number of impact categories and criteria, but by the true representation of the impact data can the risks to the aquatic environment of APIs be quantified. Research is to obtain a comprehensive overview of the gaps regarding the fate of these compounds disturb polar bear lipid metabolism.

PFAS was quantitatively the major compound group in polar bear plasma. PFAS activation of polar bear peroxisome proliferator receptor alpha (PPARA) by uptake, and determined habitat quality for individual bears. In part of the bears, we identify PFAS from the ones encountered in the impact zone; high levels of BOD, ammonia, and COD were observed in the samples. In addition, PFAS were also detected in the plasma samples from polar bears from the Norwegian archipelago, indicating that PFAS can have a potential impact on the ecosystem. The major source of APIs to the environment is human use and income countries if the level of wastewater and drinking water treatment does not increase. Untreated wastewater enters the environment via discharge into surface waters and groundwater, and is transported downstream to coastal areas.

Many chemicals used in everyday life are collected in sewage that passes through wastewater treatment plants (WWTPs). The removal efficiency of these substances varies widely, depending on the type of treatment process. However, the removal efficiency of these substances is not always consistent, and can be influenced by factors such as the type of sewage, the presence of industrial wastewater, and the presence of microorganisms. These findings are important for the design and operation of WWTPs, as well as for the development of environmental policies.
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Global sensitivity analysis in LCA of emerging technologies: Accounting for inputs' variability


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 or pilot at large scale, about 80% of environmental impacts are linked to decisions at the design phase. The application of the GSA framework to LCA models is a very helpful tool to identify these high leverage points and to reduce the uncertainty. Global sensitivity analysis (GSA) is an available tool to address this variability 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 influential 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 upgraded geothermal systems (EGS), an emerging renewable energy technology. The “baseline” GSA results provided an initial parameter ranking and the identification of the impact of the captured capacity of the Rankine cycle, the borehole depth and the number of wells as the 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 environment. 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. lifecycle inventory of the installed capacity of the Rankine cycle, the borehole depth and the number of wells as the 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 environment. 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 new pulsed-exposure early life stage test design for rainbow trout on an insecticide. Refining OECD Guideline 210 to meet the needs of EFSA Aquarium Guidance 2013

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Risk mitigation measures are a key component in defining the conditions of use of plant protection products and their links to protection goals.
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.

43 Effects of imidacloprid on freshwater outdoor microcosms in Bangladesh

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Imidacloprid is a neonicotinoid belonging to the group of SETAC priority substances, widely used in Bangladesh for a variety of crop protection. Imidacloprid may enter into the 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 community) 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 per a period of 4 weeks with four replicates per treatment. No observed effect concentrations (NOECs) for water quality variables, phytoplankton, zooplankton, macroinvertebrates, and periphyton community were identified as key parameters and included in a simplified LCA model to GSA calculations (corresponding to the baseline and 34 additional combinations, under alternative hypothesis regarding the description of the inputs’ variability. The uncertainty but its accuracy depends strongly on the description of the inputs’ uncertainty. Global sensitivity analysis (GSA) is an available tool to address this uncertainty. In this work, we propose a protocol to evaluate the effect of the selection of the inputs on the results of GSA.

44 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 exposed to drifted pesticides used at the same or adjacent 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 collected and related differences were assessed. To assess effects to the community a principal response curves (PRC) for the whole 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 scientific 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

45 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 label following the guidance of the Risk Mitigation Section (RMs) in the Product Information Document (PID) of the chemical. 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 to work on risk assessor and risk managers within 21 European countries, industry, academia and environmental regulations/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 the promotion of 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 measures. In 2016 a new 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

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

N. McDonough, European Marine Board

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 earth1. 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 and 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 Europe2. 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 Goksoy, 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

47 Red tides, Aerosols and Human Health

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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 harmful algal blooms. 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.

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

46 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 *Ichthura elegans* damsels which are exposed in microcosms to temperature scenarios. Litter-associated microbial communities were exposed to metals for 10 days while organisms were exposed, and 3) the toxicity changes over generations. 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 groups. Surfers were four times as likely as non-surfers to be faecal carriers of CTX-M-producing *Escherichia coli*. This 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 groups. Surfers were four times as likely as non-surfers to be faecal carriers of CTX-M-producing *Escherichia 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.

49 Blue Gym/Blue Health!

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

50 Marine biogenic chemicals, cell signaling and human health

M. Moog, 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 “biogenic” 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 PTEN/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., PTEIN & NF-κB), autophagy and other cell-protective processes are also affected by natural products.

51 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 safety to low-level 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.

52 Microbial pollution, antibiotic resistant bacteria, and Surfers

A.F. Leonard, University of Exeter Medical School

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 groups. Surfers were four times as likely as non-surfers to be faecal carriers of CTX-M-producing *Escherichia 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.

54 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 interactions 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 4 weeks 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 this pre-exposure phase plays an important role in determining the tolerance of zebrafish towards exposure and likely also other stressors.

56 Multi-generation toxicity of metalloid 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 metalloid mining wastes to the soil dwelling organism Enchytraeus crypticus over three generations standardized on physiological time. A metalloid-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 Cu ~9 mg kg⁻¹, total Mn ~3000 mg kg⁻¹, total Ni ~16 mg kg⁻¹, total Pb ~610 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 Lafa 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 Lafa 2.2 soil was adjusted with CaCO₃ 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 (50%+50% 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. Sin this study we demonstrate that only 30% WHC toxicity could not be reduced by any of the climatic scenarios. 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 metalloid-polluted soil to E. crypticus not only depends on the pollutant level but also on the climatic factors (air temperature and soil moisture content) to which the organisms are exposed, and 3) the toxicity changes over generations.

57 Rapid thermal evolution shapes susceptibility to ZnO nanoparticles under global warming: a resurrection study of Daphnia magna
C. Berns, KU Leuven / Department of Biology; L. De Meester, KU Leuven / Department of Biology; R. Stoks, University of Leuven / Department of Biology

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, or if 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 thermal challenge 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:N 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.

58 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 artificial forest floor conditions. The communities of microbial decomposers were exposed in microcosms to increasing concentrations of AgNPs (50 to 75,000 µg L⁻¹) and AgNO₃ (5 to 7500 µg L⁻¹) and kept at 10°C, 16°C and at 23°C. Leaf mass loss, fungal biomass and reproduction as well as microbial diversity were determined after 21 days. Also, the stress induced by AgNPs and AgNO₃ 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 AgNO₃ concentrations inhibited reproduction and diversity of fungi, particularly at the highest temperature. The activity of extracellular enzyme phenoloxidase was generally higher at 23°C. The structure of microbial communities was more related to AgNP and AgNO₃ concentrations than to temperature. Changes in temperature not only change the physicochemical properties of AgNPs, but also exacerbate the AgNPs and AgNO₃ 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

59 Modelling species sensitivity distributions for POP-contaminated wild birds using quantile-regression
R. Houssniert, J.P. Hilbers, Radboud University Nijmegen / Department of Environmental Science; M. Huijbregts, Radboud University Nijmegen; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Science

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. EC₅₀ 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 EC₅₀s (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-Cs (hazard concentration at which 5% of the species was affected) were obtained, that are suitable as thresholds in risk assessment.

60 Neurotoxic effect of mercury on Arctic Baranace goslings assessed in a controlled field study
N. van den Brink, Wageningen University / Dept of Toxicology; I.B. Scheiber,
Mercury levels in dippers at this stream were significantly higher than unregulated streams, with the exception of one recently regulated stream (Douglas Creek, British Columbia, Canada). This stream was contaminated with total mercury concentrations (Branta leucopsis) and feces (after incubation with urine and liver homogenate) which were higher in regulated streams supported higher densities and consistent occupancy of dippers 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 in regulated streams. In contrast, unregulated streams were only occupied by dippers during breeding season, and dippers were not sighted in unregulated streams during non-breeding season. These results indicate that, although exposure of organisms to mercury in terrestrial Arctic ecosystems in this study was relatively low in relation to other studies, it still results in neurotoxic effects and even effects on the organisms’ behaviour. In case of barnacle goslings this may have grave consequences, for example, interactions between parents and offspring. This warrants further studies, integrating mechanistic biochemical ecotoxicological observations with ecological relevant observations in behaviour and other fitness parameters.

61 Using American dippers Cinclus mexicanus to investigate the influence of run-of-the-river dams on mercury exposure and mountain stream food webs. V. Norbury, C.A. Morrissey, University of Saskatchewan / Biology; C.A. Bishop, University of Alberta / Electrical and Computer Engineering; J.E. Elliott, Environment Canada / Science Technology Branch

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 in regulated streams. In contrast, unregulated streams were only occupied by dippers during breeding season, and dippers were not sighted in unregulated streams during non-breeding season. These results indicate 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, for example, interactions between parents and offspring. This warrants further studies, integrating mechanistic biochemical ecotoxicological observations with ecological relevant observations in behaviour and other fitness parameters.

63 Is rodenticide use disrupting the natural regulation of vole populations? A biomathematics modelling approach. J. Fernandez-de-Simon, Laboratoire Chrono-environnement / Laboratoire Chrono-environnement; V. Baudrot, Université Bourgogne Franche-Comté; M. Courdassier, University of Franche-Comte / UMR Chrono-environnement; X. Lambin, University of Aberdeen; P. Giraudoux, Université Bourgogne Franche-Comté / Laboratoire Chrono-environnement

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 cyclical outbreaks reducing the food available for livestock and crop yields. Subsequently, some farmers respond using anticoagulant rodenticides to control voles. However, 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 however lacking. 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 this study we developed a model with two main components: (i) a population of natural 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 stabilize. We found that in Scenario 2, a pure predator-prey relationship 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 appeared in Scenario 3, with reduced vole populations due to decreased consumption of 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.

SETAC Europe 27th Annual Meeting Abstract Book
Advances in the Environmental Fate of Down-the-Drain Chemicals, including Pharmaceuticals

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 “potentially underestimated” by 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 quantitative default values for calculations of the behaviour in STP and no detailed information on primary degradation or transformation processes 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 possibility 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 sediments. 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 occurring 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 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.

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

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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 pharmaceutical ingredients (APIs) with suspected or known reproductive effects. In 2016 EMA launched an ERA concept note consultation on assessing ERA needs and ERA guidance on the ERA of human medicinal products. This presentation will describe the EMA ERA guidance in detail and address some of the key scientific issues associated with the current EMA ERA guidance, EMA concept note and proposed ERA guideline revision drawing from experiences gathered over the last ten years. All observations and recommendations will be made using empirical data generated to support marketing applications. This includes new data from the scientific literature for the ERA Phase 1 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.

Using HILIC stationary phases to predict fate and behaviour 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 (Kow) is widely used as a hydrophobicity parameter in quantitative structure-activity relationships to predict the environmental fate, present and effects of non-ionic organic compounds. However, Kow 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 biodegradation of different surfactant classes. We tested the performance of selected surfactants on different stationary phases in liquid chromatography (e.g., C18, HILIC or weak ion-exchange phases) to study the three basic sorptive interactions of surfactants (i.e., hydrophobic, hydrogen-bonding and electrostatic interactions). From the resulting retention times, a logarithmic capacity factor could be calculated which represents a combination of the hydrophobicity, k’ on hydrophobic phases, k’W on hydrogen-bonding interactions, and k’E for electrostatic interactions. The logarithmic capacity factors determined for alcohol ethoxylates with both C18 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.

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 physiochemical 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 influent rate, the plant capacity, and the influent concentrations, Kow, and Koc. Collinearity and heterogeneity tests led to the choice of the Kow, 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 Kow 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, specific surfactant interaction 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.

Mapping risk assessment challenges for HPC ingredients: a chemical space in 3D

T. Goun, Unilever / Safety and Environmental Assurance Centre; O. Price, C. Rendal, Unilever / Safety and Environmental Assurance Centre SEAC; R. van Egmond, Unilever

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 (cleaning, 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 lipophilic. Neutral organics, ionic surfactants and ionically interacted 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 (>2000) and discuss implications towards assessing behavioural properties. 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)

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

D. Hug Peter, E. Castella, University of Geneva / Department FA Forel for

From biodynamic modelling to the field: Cd and Cu availability for food could be an important factor for determining the bioavailability of Cu, and we did not differ significantly among the three types of metal-rich material. Based on the specific modelling and laboratory research challenges that risk assessors face in dealing with HPC ingredients.

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A laboratory experiment was conducted to investigate the main uptake route and accumulation of cadmium in brown crab Cancer pagurus from different types of material. 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/L wet weight 108Cd in feed. We used gavage feeding to standardize the feeding of the crabs (76 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 (2, 7, 14, 21, 30 and 42) and the depuration phase (2, 4, 7, 21, 35). We analyzed hepatozellapancras, gills and hemolymph 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.

Use of reverse-isotopic labeling of Nitzschia stains to understand dieterbole Cu bioavailability in receiving waters affected by contamination and remediation of acid mine drainage


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 soils (Nitzschia stagnalis) that were fed metal-rich material collected from the sediment. The isotopic signature of the nitrates was retained by cultivation of Nitzschia from metal-rich water for five weeks. Various amounts of metal-contaminated material were mixed with diatoms (Nitzschia paeona) to produce a series of dieterbo-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 remediation of NFCC begins in January 2017. Ongoing research is underway to characterize physical changes of the sediment compositions and bioavailability throughout the remediation process.

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

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Sacrificial zinc anodes are used on millions of vessels worldwide to provide corrosion per vessel and anode weights from a survey of boat owners. Similar estimates for wastewater treatment works suggest 0.062 to 0.151 kg/yr of zinc is input into the estuary, which is considerably lower than anode inputs. The MAMPE 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 corrosion products are a major source of zinc to estuaries, with similar sources such as waste water treatment works. However, water and sediment sample collection on the Hamble estuary, which has 3000 pleasure vessels indicates zinc anodes could be the main source of zinc 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 6.958 kg/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 0.151 kg/yr of zinc is input into the estuary, which is considerably lower than anode inputs. The MAMPE 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 µg/l. Observed total dissolved zinc concentrations ranged between 3 and 35 µg/l, with the majority of samples between 5 and 20 µg/l. Concentrations above the boat moorings and in the Hamble river itself, were measured to be below 8 µg/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 4.52 µg/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 226 µg/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.

72 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 3000 pleasure vessels indicates zinc anodes could be the main source of zinc 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 6.958 kg/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 0.151 kg/yr of zinc is input into the estuary, which is considerably lower than anode inputs. The MAMPE 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 µg/l. Observed total dissolved zinc concentrations ranged between 3 and 35 µg/l, with the majority of samples between 5 and 20 µg/l. Concentrations above the boat moorings and in the Hamble river itself, were measured to be below 8 µg/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 4.52 µg/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 226 µg/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.
effluent were collected and separated into particulate (i.e. > 0.45 µm), colloidal (i.e. 1 kDa to 0.45 µm), and truly dissolved (< 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 (46%) and Zn (68%) were removed 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)

74 Not relevant? The significance of non-regulated pesticide metabolites H. Banning, Federal Environment Agency (Umweltbundesamt); W. Koenig, Federal Environment Agency (UBA); A. Mueller, Federal Environment Agency / IV 1.3; C. Pickl, Federal Environment Agency

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 production costs and forced acceptance by customers. 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 the German pesticide monitoring programme 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 continue 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 µg L⁻¹ and in 13% above 1 µg 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 µg L⁻¹ there are only 13 parent substances, detects with values above 1 µg L⁻¹ comprise only 8 parent substances, three of which 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 (NG01). 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 initiatives are highly appreciated, but should be extended to all aquifers and not be limited to drinking water abstraction areas.

75 Significant processes for calculating environmental concentrations of plant protection products in the sediment compartment of FOCUSsw scenarios D. Patterson, Syngenta; A. Strehmel, B. Erzgraeber, BASF SE; K. Hammel, Bayer Crop Science Division / Environmental Safety

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 the parent and metabolites over similar 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 for the distribution of grain sizes and thickness of the water column. In order to access transport and deposition dynamics of suspended sediment 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 for the distribution of grain sizes and thickness of the water column. In order to assess transport and deposition dynamics of suspended sediment 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 for the distribution of grain sizes and thickness of the water column. In order to access 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 for the distribution of grain sizes and thickness of the water column. In order to access 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 for the distribution of grain sizes and thickness of the water column. In order to access 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 for the distribution of grain sizes and thickness of the water column. In order to access transport and deposition dynamics of suspended sediment on the
that are based on details of exposure peak characteristics. In 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 Scenarios Water and their mod (ref to nanotechnology field) have to the interpretation of the results and the use of multi-year exposure data in (higher-tier)
risk assessments is still under discussion. 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 possible indications of the 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)**

**79 Toxicokinetics of silver nanoparticles in the freshwater snail Physa acuta**

P. Silva, University of Aveiro; C. van Gestel, R. Verweij, VU University Amsterdam / Ecological Science; S. Loureiro, Universidade de Aveiro / Biology

The fate of silver nanoparticles (NPs) is a relevant subject for aquatic risk assessments. AgNP60 were placed into 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 (3-8 nm, 60 nm, 50 nm, Ag2S) including one silver sulphide NP (Ag5S-NP), and their ionic counterpart as silver nitrate (AgNO3) were tested. The organism 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 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 deputation kinetics was more similar to that of the smaller Ag-NPs. This may demonstrate similarities in solution chemistry and/or uptake of the organism. Furthermore, the very slow elimination upon exposure to small Ag-NPs and ionic Ag (AgNO3) could lead to the potential transfer of Ag to higher trophic levels in the aquatic ecosystem.

**80 Toxicokinetics of silver nanoparticles in the meadow Tenebrio molitor**

Z. Khodaparast, University of Aveiro / Biology; C. van Gestel, R. Verweij, VU University Amsterdam / Ecological Science; S. Loureiro, Universidade de Aveiro / Biology

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 mealtreworms. The soil was spiked with 100 mg Ag/kg dry soil with different silver AgNPs (3-8 nm, 60 nm, 50 nm, Ag5S) and AgNO3. 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 mol imagining transitively (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 NP-38nm, NP60nm and Ag+ respectively. Bioaccumulation factors (BAF) showed that AgNP60 were more accumulated in the mealworms compared to AgNP-38nm and Ag+. Moreover, based on the estimated inert fractions (0.32, 0.14, and 0.57 for NP-38nm, NP60nm and Ag+), AgNP60 were more retained by the mealworms compared to other studied Ag forms, while Ag+ and AgNP-38nm displayed an insignificant difference. This may be due to higher Ag+ release from smaller silver nanoparticles. The analysis of the mols of the mealworms showed that the amount of silver which can be eliminated by molting is insignificant. Toxicokinetics in mealtreworms in aquatic environment, depending not only upon the composition of the particles but their interaction with key biological macromolecules is driven by the total number of Persistent Organic Pollutants (Globo-POP) was used to assess the global over-representation of PFOA and PFOS also exists in the toxicological literature, for PFAS other than PFOA and PFOS, even in recent publications. A similar combination of both exposure pathways resulted in a distinct mortality of ~30%,

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

M. Baccaro, Wageningen University / Toxicology Department; A. Undas, RIJKL Inst. Of Food Safety WUR / Business Unit Contaminants Toxins; H.H. van den Berg, N. van den Brink, Wageningen University / Dept of Toxicology

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 Ag-NPs in organisms quantified NPs by analysing a portion 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 AgNO3. 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 (k1) and elimination (k2) kinetics parameters: K1 is 0.015 and 0.039 gsoil g−1 day−1; K2 is 0.070 and 0.048 day−1, for Ag-NPs and Ag+ respectively. This is the first study that provides kinetics 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 NPs may not be straightforward at all.

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

S. Lüderwald, Universität Koblenz-Landau / Institute for Environmental Sciences; T.C. Schell, Universität Koblenz-Landau / Institute for Environmental Sciences; F. Seitz, Inst. for Environmental Sciences / Institute for Environmental Sciences; R. Rosenfeldt, Universität Koblenz-Landau, Institute for Environmental Sciences / Institute for Environmental Sciences; K. Newton, University of Koblenz-Landau; V. Schaffers, E. Schaffers, R. Schaffers, Koblenz-Landau / Institute for Environmental Sciences; M. Bunde, M. Schaffers, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment

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 (TiO2) 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 – whether waterborne or dietary – as a risk factor is often under-considered. The purpose of this study was to uncover the influence of these exposure pathways on the effects caused by TiO2 and nAg. Therefore, for each NP type an individual 30-d long bioassay with the benthic amphipod shredder *Gammarus fossarum* was conducted, using a 2x2 factorial test design. The factors comprised of the presence or absence of the NP (0/95; −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...
and ionic Ag (AgNO₃) could lead to the potential transfer of Ag to higher trophic levels. Depuration kinetics was more similar to that of the smaller Ag-NPs. This may be due to higher elimination rate constant (k₂) values when compared to the animals exposed to silver sulphide NP (Ag₂S-NPs), and their ionic counterpart as silver nitrate test compounds at a nominal concentration of 10 µg Ag.L⁻¹ and then transferred to the soil. The soil was spiked with 100 mg Ag/kg dry soil with AgNPs in mealworms. The soil was dried, digested and analysed for Ag by graphite furnace Atomic Absorption spectrometry. Kinetics of Ag-NPs and ionic Ag in the snails were straightforward analytical methods. Most of the ecotoxicological studies on uptake and elimination kinetics of the different Ag forms in organisms is considered essential for environmental risk assessment. Their complex behaviour, properties and interactions with environmental factors are poorly understood. The purpose of this study was to uncover the influence of these exposure pathways on the effects caused by nTiO₂ towards nAg (up to 30%). In contrast, the dietary exposure towards ionic Ag 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.

83 Routes of uptake and bioaccumulation of cerium oxide and silver nanoparticles depend on their fate in sediments R. Cross, University of Exeter; C.R. Tyler, University of Exeter / Biosciences College of Life and Environmental Sciences; T.S. Galloway, University of Exeter / Department of Biosciences

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 time spans. A crucial question is: how long do NPs stay in the system 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 investigated the production of oxidising products or 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 (CeO₂-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 CeO₂ or Ag NPs. Results indicate that NPs which associate with the solid fraction of the sediment and do not dissolve within the sediments (CeO₂-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.

84 The burden of persistence: towards an understanding of PFAS exposure C.A. Ng, University of Pittsburgh / Civil & Environmental Engineering; A. Jordan, E. Yilmaz, University of Pittsburgh / Department of Civil and Environmental Engineering

The US EPA recently published a Drinking Water Health Advisory for perfluorooctanoic acid (PFOS) and perfluorooctane sulfonic acid (PFOSA) 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, we speculate that the perfluoralkyl 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 classify 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 PFOS and PFOSA, even in recent publications. A similar over-representation of PFOSA 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 extrem persistence and high bioavailability of PFAS, it is critical that we develop a better understanding of their toxic impacts. This is especially true for the case 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.

85 The environmental emissions, global distribution and risk assessment of F-53B B. Li, L. Li, J. Liu, Peking University

The environmental releases and concentrations of 6.2 chlorinated polyfluorinated ether sulfonate (6:2 CI-PFES) 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 during the period of 2006-2015 based on the bottom-up emission estimation method and the analysis of statistics data from Chinese metal plating sector. A global fate and risk 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 regions 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 (EUROSE 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 BCF, 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.

86 Degradation of poly- and perfluoroalkyl substances (PFASs) from industrial wastewaters on BDD electrodes A. Urtiaga, B. Gomez-Ruiz, Universidad de Cantabria / Dep Ingenierias Quimica y Bioloquimica; S. Gomez, Universidad de Cantabria (VAT N° ESQ-3918001-C) / Chemical and Biomedical Engineering; A. Colin, V. BOITEUX, X. DAUCHY, ANSES

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 PFOS and PFOSA [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 perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid, n,2 fluorotelomer carboxylic acids, n,2 unsaturated fluorotelomer carboxylic acids, n,2 fluorotelomer sulfonic acids, perfluorooctanoic 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 SPFAS 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/cm² for 10 hours of operation. Furthermore, the TOC removal rate was 91%. It was found that the major fluorotelomer contents in the feed were initially converted into perfluorocarboxylic acid, which was further oxidized into shorter chain PFCAs. A defluorination factor of 126% elucidated that other unknown PFASs were being degraded during the electrochemical treatment of the wastewater. References [1] Urtiaga A, Fernandez C, Gomez-Lavin S, Ortiz I. 2015.Kinetics of the electrochemical mineralization of perfluorocarboxylic acid on ultrananocrystalline boron doped diamond electrodes. Chemosphere, 129:20-26. [2] Dauchy X, Boiteux V, Colin A, Harzheim L, Muniz JL, Gross A. 2018. Electrochemical- and perfluoroalkyl substances (PFASs) in the wastewater treatment plant of a fluorochrome manufacturing facility. Sci. Total Environ. 576: 549-558.

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Development and assessment of alternatives to long-chain PFAS

The family of perfluoralkyl substances (PFASs) 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 national or regional level. The major EU, US and Japanese fluorotechnology manufacturers have over the last decade developed alternatives to a specific class of per- and polyfluorinated compounds referred to as long-chain PFASs. 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.

88 TBD

Input/output and Hybrid Life Cycle Assessment for supporting the assessment of production and consumption patterns
89 Hybridizing Ecoinvent and Exiobase with streamlined methods and open-source software
G. Majeau-Bettez, M. Ager, CIRAIG; Polytécnica Montreal; R. Wood, NTNU; M. Margni, CIRAIG - École Polytechnique de Montréal / Mathematical and Industrial engineering; A. Stromman, NTNU; R. Samson, École Polytechnique de Montréal / Department of Chemical Engineering

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-EEOIO 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 complex cost coverage and systematically underestimate requirements for services and infrastructures, even studies that hybrize 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.

90 Increasing the regional resolution of the Ecoinvent LCI database with GMRO data
A. Haas, Institute of Environmental Engineering, ETH Zurich / Chair of Ecological Systems Design; S. Pfister, ETHZ / Chair of Ecological Systems Design

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 multiregional input-output databases such as Ecoinvent and 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 regions in Europe based on Exiobase data. 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 GMRO 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.

91 EU Life cycle indicators - based on hybrid LCA
M. Schmidt, 2.0 LCA consultants; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

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 EU/28. The total GHG emissions calculated using the territorial-based approach in the EU28 are 4.7 billion tonne CO2-eq. (9.3 tonne CO2-eq. per capita). Using the consumption-based approach, the results increase by almost 25% to 5.8 billion tonne CO2-eq. (11.5 tonne CO2-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 categories (GMRIO database) at present. The data behind 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.

92 BONSAI: from vision to implementation
M. De Rosa, Arhus University / Agroecology; S. Merciai, M. de Saxcé, B. Weidema, BONSAI

The BONSAI 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 features 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 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 it 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.

93 Regional and temporal variation in environmental impacts from household consumption

V. Zeller, Université Libre de Bruxelles; M. Degrez, Université Libre de Bruxelles / Materials engineering, characterization, synthesis, and recycling (4MAT)

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) variations of household consumption is difficult, due to the low availability of regional IO models. However, such an assessment is particularly relevant when regional differences 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 substantial resolution for Belgium (Brussels, Flanders and Wallonia). The regional household expenditure for 2003, 2007 and 2010 indicates the consumption of 81 products groups, separated 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 inhabitant 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 environmental 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 homogeneous 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 between Flanders and Wallonia is observed in the majority of the midpoint categories (such as food, clothing, housing, etc.) and direct impacts. The small differences that were found represent specific urban conditions (housing and transport) that cannot be transferred on the rural regions.

The role of eco-toxicology in fisheries science and aquaculture (I)

94 Controlling the effect of aquaculture on the environment

-Oalmaid, Marine Harvest ASA

We work to secure the future of our farming 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 world’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 vertebrates, the brown trout is one of the most important family 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 by 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: in v1. 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.

51 Marine 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 and shellfish - 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 farmers more off-site thus limiting environmental impacts and allowing for a sustainable growth of the industry.

52 Emerging contaminants and toxins in aquaculture: how modern analytical tools may aid to ensure food and environmental safety

-L. Vanhauweck, 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 utmost 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 algacides 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 algacides, frequently applied to control aquatic weeds, algal blooms and fouling organisms. As such, a variety of biological active and possibly toxic chemical substances 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 bio-toxins may strongly affect food growth rate, death rate and food safety. Within 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 metabolism of bio-active substances by (edible) organisms, 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 key process of contaminant analysis and adequate monitoring will be demonstrated using practical examples. From this, current shortcomings and future needs for a better food safety warranted and sustainable production will be proposed.

Contaminant control and development in farmed salmon- industry data

-Oalmaid, Marine Harvest ASA

Marine Harvest is one of the largest seafood companies in the world and the world’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 are present in 28 countries and are present in 24 countries. We aim to be an integrated provider of proteins from the
established in the EU. They pose to consumers?

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. Our 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. In

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

I. Katsiadiaki, 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 (E2) in mollusc tissues is an area of growing 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 E2 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 E2 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 radioactive label that was in c. 40 mL of water in one hour. The rate of uptake of radioisotopes could not be saturated by the addition of up to 25g/L non-radioisotopic T and E2 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 radioisotopes (>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. E2 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 biodistribution associated with 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)

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

J. Wheeher, Dow Agrosciences

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, semiquantitative 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.

100 Ecological risk assessment of chemical mixtures

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 species may vary, the results of exposing different species to a chemical under varying conditions thereof. Approaches to deal with potentiation (synergism) or attenuation (agonism) 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.

101 Endocrine disruption: A toxicologist’s perspective

S. Munn, European Commission

Endocrine disrupting substances are considered a major threat to the environment. In the EU, three strategies have been proposed for implementing a more comprehensive approach to endocrine disruption: i) an integrated risk management approach, ii) a health-based approach, and iii) a scientific approach. The scientific approach is based on a combination of ecotoxicological and environmental risk assessments, and the establishment of endocrine disrupting chemical (EDC) screening and definitive testing in multiple taxa. These traditionally are outside of the scope of 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.

102 Endocrine disruption: An ecotoxicologist’s perspective

J. Wheeher, 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 geographical 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 genomic models for specific activities (e.g. amphibian metamorphosis and thyroid activity) or particularly sensitive indicators in screening batteries (e.g. fish for steroidalogenesis). 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.

103 Panel discussion

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

104 Influence of temperature and moisture on toxicity of propiconazole and chlorantraniliprole to earthworm Eisenia fetida
B.K. Hackenberger, G. Palićan, L. Lonšar, Department of Biology University of Osijek, D. Hackenberger, Department of 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 after exposure to soil extraction. The results of the 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.

105 Chlorpyrifos-induced oxidative damage is reduced under warming and predation risk
L. Janssen, K. U. Leuven, 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 organisation. We aimed to study 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 (sod, 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 cope 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 overall oxidative damage, the pesticides 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.

106 Strong differences between two congenic specific in sensitivity to pesticides in a warming world
S. Stoks, J. Verheyen, University of Leuven / Laboratory for Aquatic 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 sensitivity increase). 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 did not depend on warming, 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.

107 Sequential pesticide exposure increases toxicological sensitivity of crustaceans R. T. Rasso, Helmholz centre for environmental research - UFZ / System Ecology, 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 fenvalerate compared to fish 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.

108 Combined effects of water scarcity and an insecticide on freshwater zooplankton communities: a microcosm study
A. A. Sanchez, IMDEA Water Institute / Aquatic Ecotoxicology; I. Lopez, 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 impacts 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 stressors factors: increasing water temperatures and droughts. Twenty-seven microcosms were stocked with pond water, sediment, and a homogenised planktonic microalgal. 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. Insecticides 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 rotiferia. 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**

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

L. Salaberry, 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, BioTix; T. Nordtug, SINTEF Materials & Chemistry / Environmental Technology; A. Olsen, Norwegian University of Science and Technology.

Increased ice coverage is pushing 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 hours exposure 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.

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

P. Blévin, S. Tartu, O. Chastel, CEBC CNRS & Université de la Rochelle UMR 7372; P. Bustamante, Université de La Rochelle / Littoral Environnement et Sociétés LIENSs; C. Parenteau, CEBC CNRS & Université de la Rochelle UMR 7372; H. Hessel, NorCeST Recherche Centre NUII; F. Angelier, CEBC CNRS & Université de la Rochelle UMR 7372; H. Ellis, University of San Diego; G. Gabrielsen, Norwegian Polar Research Institute

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, perfluoropolyalkyl substances (PFAs), and mercury) with BMR and circulating total THs (thyroxine (T4) and triiodothyronine (T3)) in arctic breeding adult black-legged kittiwakes (Rissa tridactyla). Our results indicate a negative relationship between chlorodane mixture (YCHLs) 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 T3 were negatively related to YCHLs but not to PFTRA. Organochlorines and PFAs may therefore disrupt fine adjustments of BMR during reproduction in adult kittiwakes and this may cause fitness penalties. Importantly, highly lipophilic organochlorines and highly proteinophilic PFAs 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 assess a large variety of contaminants which can act in an antagonistic manner.

111 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 phenomenological mismatch between processes and their prey availability may affect key 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 ring seal (sea seal) breeding season (spring to early summer). Consequently, 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 PFAs) 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 indicators 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.

112 Are snow bunting (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 addition, emissions of potentially toxic pollutants to the local environment are 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, emissions from the coal-fired power plant and other urban activities in Longyearbyen, Svalbard. Thus, feathers were sampled from adult-nestling 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 Zr in adult bird feathers indicates the possibility that geochemical 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 VINTERSPUR project ‘Reproductive biology of the snow bunting (Plectrophenax nivalis)’ funded by the Svalbard Environmental Protection Fund.

113 Causes and consequences of mercury exposure in an Antarctic seabird

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The study contributes to describe the differential vulnerability of aquatic ecosystems to effects and population/community recovery is still undergoing. The results of this study are important, highly lipophilic organochlorines and highly proteinophilic PFASs become apparent. BMR during reproduction in adult kittiwakes and this may cause fitness penalties. Tridactyla 24 rate in a contrasted manner 110 depuration period. This slow depuration of PAHs in lipid rich Arctic copepods pyrene and chrysene showed up to approximately 100 % retention after a 120 h findings suggest that the physiological effects of short term exposure to components. Particular focus is on bioaccumulation kinetics, oil component kinetics of selected produced water components and oil droplets and their effects on bioavailability in Water, Soil and Sediment (III).

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

114 REEchangE-Rare Earth Elements in a Changing Environment H. Tian, Hamburg University of Applied Sciences/University of the West of Scotland; F. Beinwolks, Hamburg University of Applied Sciences; A. Hursthouse, University of the West of Scotland / School of Science; S. Heise, Hamburg University of Applied Sciences / Life Sciences 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. 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), exemptly performed for German 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 wasteland and specialised industries as prominent sources of emissions. (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 Alivibrio fisheri and Rhabdophilus subcapitatus so far are in the same range as literature data, and show a higher toxicity of Gd compared to La. Concerning the contribution heavy metals toxicity also appears to be in a comparable range of concentration. Organism ECoGd ECoLa A. fischeri (this study) 23.45 µg/ml 11.76 µg/ml P. subcapitata (this study) 0.945 µg/ml 11.32 µg/ml Key Words: Rare Earth Elements, Substance Flow Analysis, Aquatic Ecotoxicology, HERRMANN, H. NOLDE, J. BERGER, S. HEISE, S. 2016. Aquatic ecotoxicology of lanthanum – A review and an attempt to derive water and sediment quality criteria. Ecotoxicology and Environmental Safety, 124, 213-238.

115 Derivation of soil threshold concentrations for arsenic: evaluation of ecotoxicological data for several species of soil organisms J. Brack, e.c.t. Ökotoxikologie GmbH, J. Römcke, A. Schefczyk, ECT Ökotoxikologie GmbH; S. Jänsch, ECT Ökotoxikologie GmbH; D. Rückenkamp, E. Fries, Federal Institute for Geosciences and Natural Resources; E. Giese, J. Uerrmann, Federal Environment Agency; K. Oorts, ARCHJE The German Federal Soil Protection Act (1998) 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. A further step is the development of a more representative way for organic matter content etc. or using other metal extractions, such as 0.01M CaCl2, 1M NH4NO3, 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. Soils covering a wide range of Central European soil properties were chosen and spiked with soilardised arsenaibic dib Stephatic. In these soils microbes, plants and invertebrates were tested, according to the ISO standard guidelines. Test results (given as EC50 values based on aqua-regia extractable As concentrations) are compared with different soil properties (pH, organic carbon content, Al-, Fe-, and Mn- content, etc.) to bioavailability of As in five distinct representatives 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 EC50 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.

116 Improved comparative toxicity potentials of 23 metallic elements in soils: addressing solid- and liquid-phase speciation in environmental fate, exposure, and effects M. Baban, Technical University of Denmark; M. Hjuljebjeg, Radboud University Nijmegen; M. Hauschildt, DTU Management Engineering / DTU Management Engineering 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 alkali 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, Ti, 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 Sr(III). The largest variability in the CTPs, by up to 6 orders of magnitude, was estimated for elements which precipitate as hydroxides, nitrates, carbonates, chlorides (Cr, Fe, Cu, Hg), CH3C, iron and manganese to 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.

117 The effect of ageing on lead toxicity to the soil invertebrate Enchytraeus crypticus L. Zhang, VU University Amsterdam / Animal Ecological, C. van Gestel, VU University Amsterdam / Ecological Science 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(NO3)2) sorption may increase with time, while for a poorly soluble form (e.g., PbSO4) dissolution may lead to an increase in availability with time. 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(NO3)2) and lead oxide (PbO) in the potworm Enchytraeus crypticus in Lufa 2.2 natural soil. Worms were exposed to Pb shortly after oxidising the soil and under anaerobic conditions to test whether MnP, lignin peroxidase and reproduction after 21 d exposure were related to total, 0.01 M CaCl2 extractable and

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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 slightly for PbO. LC50 and EC50 values for the effect on enchytraeid 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 0.05 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 preserved from internal Pb concentrations in the watercourses next to pome fruit orchards in the Netherlands. This paper describes how the 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. Improving the understanding of behavior and fate processes through simulations of modelled exposure scenarios for the urban environment is further assessed. SETAC DRAW aims to find a consistent, realistic and transparent approach for spray drift exposure assessment, and the use of models will be an important component of this.

120 Options for the spatial statistical population of the exposure assessment goal for aquatic organisms at EU level

J. Boesten, Wageningen Environmental Research

Assessment of risks for aquatic organisms is an important aspect of the environmental risk assessment of pesticides in western countries. This risk assessment is based on a database of aquatic organisms that are sensitive to pesticides, but only slightly 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 preserved from internal Pb concentrations in the watercourses next to pome fruit orchards in the Netherlands. This paper describes how the 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. Improving the understanding of behavior and fate processes through simulations of modelled exposure scenarios for the urban environment is further assessed. SETAC DRAW aims to find a consistent, realistic and transparent approach for spray drift exposure assessment, and the use of models will be an important component of this.

118 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 usual water chemistry of waters associated with laterite geology has led to the evolution of aquatic organisms that ionoregulate differently than non-laterite species and that 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. They are formed by extended weathering under tropical climatic conditions 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-laterite 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)

119 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 downward 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 (PECs) 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.

121 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 whole 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 transparent approach for modelling spray drift exposures, and the use of models will be an important component of this.
Mitigating pesticide runoff in an agricultural catchment
L Joris, N. Desmet, W. Boënne, P. Seuntjens, VITO; K. Koopmans, D. Bylenselm, pefrut; K. Vandraele, Watering Staten-Tinted
Pesticide concentrations in rivers generally have a very dynamic signature and are strongly dependent on time and space. The dynamics in the time-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 pesticide management. It 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 buffer strips), in connection with drainage pathways (i.e. grazed waterways) and the creation of (small) sediment deposition 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.

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

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 about the influence of CNMs 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 indicated that MWCNTs exposure under lower pH levels (7.6) showed similar or opposite 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 the decrease in lipid peroxidation (LPO). Keywords: Multi-walled carbon nanotubes (MWCNTs), pH variation, Ruditapes philippinarum, biochemical alterations
IDAEA-CSIC / Environmental Chemistry; G.F. Schirinzi, IDAEA-CSIC; D. Barcelo, IQAC-CSIC / Dept Environmental Chemistry

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 reveal the ability to modulate the toxicity of other co-contaminants. Plastic debris pollution has been recognised as a significant problem in marine environments. However concern has been focused on microplastics (MPs), 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 MPs 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, minor increases in free amino acids were observed 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, steric, 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 confident estimation of 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 in several regression models in plaquelet arrays. 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 the specific attention is devoted to those features which are most predictive of toxicity.

Persistence & Biodegradation Assessment

The impacts of light and season on isopryrazam degradation in river

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Industry uses tests designed by the Organisation of Economic Cooperation and Development (OECD) to assess transformation potential of the mixture 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 1C-isopryrazam, 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 (Welllesbury, UK), and both water and sediment microcosms were incubated under light (16 hour light/8 hour dark cycle) conditions. Isozopryrazam 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 isopryrazam 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 isopryrazam degradation rate. Profiling of microbial communities using 23S RNA 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
H. Birch, DTU Environment / Department of Environmental Engineering; R. Hammershøj, P. Mayer, Technical University of Denmark / Department of Environmental Engineering

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 quality and quantity 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 8 chemicals including paraffins, napthenics and aromatic hydrocarbons from C8 to C20. Passivated carbon (PC) was added to the PW compounds in the PW 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 hydrocarbons. In lakewater and seawater, less test chemicals were degraded within the test duration compared to the activated sludge filtrate. The OSPAR Commission regulates operational discharges such as produced water (PW) from the North Sea offshore industry to the marine environment, classifying PAHs (2- to 3-ring), as well as C0-C9 APs. Larger PAHs (4- to 5-ring) were observed in dispersions in natural seawater, but not sterilized controls. Analyses of PW compounds in the PW were separated between a prevalent (up to 167 µg L-1). Both the overall removal (1190%) in wastewater

α-reductase (5α-3β-reductase) favors conversion of androgens to estrogens, which has been hypothesized to contribute to the increased risk of breast cancer in women with higher serum levels of these hormones. In this study, the authors evaluated the potential role of α-reductase 2 expression in breast cancer risk. They performed an mRNA expression analysis of α-reductase 2 in a cohort of breast cancer patients and compared it with a control group of healthy women. The results showed a significant upregulation of α-reductase 2 expression in breast cancer tissues compared to healthy controls. This finding suggests a potential link between increased α-reductase 2 expression and increased breast cancer risk.

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in seawater, using low concentrations of PW compounds. One method was based on immobilization of poorly water-soluble compounds to hydrophobic adsorbents (Fluorotex(TM)). This method enabled biodegradation studies both on the adsorbent surfaces and in the surrounding water. Three APIs with suspected PBT properties were tested in the method (20°C, 64 days), and results compared to data from an OECD flume test. OECD and NWTP compounds 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 Fluorotex 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 PAs and APIs showed >90% biotransformation of naphthalenes and smaller PAHs (2- to 3-ring), as well as CO-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 hydrophobic adsorbents (Fluorotex) during biodegradation experiments, and to characterize metabolites in relation to toxicity ranges during biodegradation.

132 Degradation of synthetic fluorescent dyes in land spreading of MWWTP sludge


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 14C-labelled acrylamide in a downscaled procedure with the labelling positioned along the carbon-carbon backbone of the amide units. The 14C-labelled polymer met the product specifications of commercially available polymer used in sludge dewatering polymer. The 14C-polymer was used to dewater digested sewage sludge from a local MWWTP in a standard way and the dewatered sludge containing the 14C-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.

133 Poster spotlight: MO142, MO143, MO144, MO145

Hazard and risk assessment of human pharmaceuticals in the environment

134 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-retrovirals (e.g. nevirapine and zidovudine) and co-trimoxazole (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 matrices. 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 dumping sites 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.

135 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 Comparison, 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, 363 APIs were found to target at least one protein in all tested fish and 5 APIs had orthologs in across this group. For protostomes 354 APIs lack orthologous gene targets, meanwhile invertebrate deuterostomes may only lack targets for 167 APIs suggesting that Daphnia 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.
function later in life, without overt toxicity or morphological changes. 5uR 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 5uR in gastropod biology of molluscs if we are to protect them from pollution in the environment. The ‘Guideline on the environmental risk assessment of medicinal products for the built environment.’ 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 substantiate 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. In any case, 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 for 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

#### 137 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 β₁-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 significantly 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 mammalian Cyp2D6. However, 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, Küster 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.

#### 138 Review of the EU guideline on ERA of HMIs

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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 (EMAPA/CHMP/SWP/4466/2010, rev. 1). In 2016 the European Medicines Agency highlighted a re-evaluation of the guideline. The ‘Guideline on the environmental risk assessment of medicinal products for human use’ (EMEA/CHMP/SWP/65429/2016) which was also subject for public consultation.

Eight topics 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 addressed in the concept paper is a review of the tiered approach strategy and triggered for further harmonisation 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 substantiate 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. In any case, 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 for 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.
using the principles of the “element method for cost control” and a subdivision 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. More research is required to highlight the impact of the layout of the
neighbourhood layout and built density to reduce the environmental impact of the built environment.

141 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 land use services or land uses provided by land planning scenarios that are specific to the territory and 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.

142 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 essential 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 as it is only an external perspective that are modelled 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 2010-2010 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 output flows of materials and energy for each building construction, use phase, renovation and demolition. Then, environmental 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.

143 An application of Spatial Territorial LCA to agriculture in Luxembourg: use the TMML R package
S. BOURRELLY, Unité mixte de recherche ESPACE; A. Marvuglia, Luxembourg Institute of Science and Technology (LIST) / Resource Centre for Environmental Technologies (CRTE); Resource Centre for Environmental Technologies (CRTE) Nitschelm et al. (2016) recently developed a method termed spatialised territorial LCA (STLCA) that combines spatialized LCA and territorial LCA to study land use planning scenarios 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 (TML) 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 baseline functions for statistical cartography and spatial clustering allowing a better 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 ecoctoecology in fisheries science and aquaculture (II)

144 Do fisheries practices increase contaminant mobilisation from sediments?
J. Allan, Norwegian Institute for Water Research; C. Bradshaw, Stockholm University / Departments of Ecology Environment and Plant Sciences; T. Tjensvoll, Stockholm University; K. Næs, Norwegian Institute for Water Research; H. Nilsson, Swedish University of Agricultural Sciences / Institute of Marine Science
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 km2 of the world's seafloor every year, 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 m3 sediment plume containing 90 ton contaminated sediment; i.e. 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/Fs 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.

145 Environmental contaminants in wild fish from the North East Atlantic
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 that are so far, thereby impacted by sources from local and far-lying 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 region. 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 harengus), mackerel (Scomber scombrus), cod (Gadus morhua), saithe (Pollachius virens) and haddock (Melanogrammus aeglefinus). However, our research projects 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 (Reinhardtia 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

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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 commercial 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 turbot. 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 seafood 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 TW/PWTW values, mercury will be the limiting factor for high seafood intake for filament 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 TW for methyl mercury. While for other species such as cod from the Baltic Sea, it would take 16 meals per week to reach TW. 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.

146 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 first 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 contamination. 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.

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147 Has tributyltin contamination reduced catches of the brown shrimp Crangon crangon?

K. Cooreman, ILVO

148 Discussion

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

149 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, 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 a 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 biological 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 fulfill their 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.

150 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 the 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 delodinase (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, USEPA policy.

151 The zebrafish embryo model for human risk assessment: a SWOT analysis

S.J. Van Cauwen, 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,
152 The fish embryo model for the environmental assessment of chemicals
M. Leonard, IOREAL SA

There is a worldwide trend to promote the use of alternative 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 (ZoFET), 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 Embryotoxicity test. In addition, when sub-lethal endpoints such as development 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.

153 Panel discussion

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

154 Development of an AOP describing effects of narcotics on membrane-bound mitochondrial processes in fish
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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 antipyrine using a passive dosing method. To assess potential detoxification pathways, we hypothesized mitochondrial-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 concentrations, ETC activity decreased 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 Kow dependent. This relationship suggests that the 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.

155 An improved spgl1-gfp medaka assay for the detection of (anti-)androgens - the RADAR assay
A. Tindall, G. Chritiens, Watchfrog S.A.; I. Katsiaki, Cefas / Environment and Animal Health; B. Demeneux, 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 through in vivo assay for androgen axis disruption. We developed a transgenic medaka line harbouring a portion of the spg1n 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 homoyzoygous fry harbouring a transgene coding for a red eye marker cassette on the same chromosome as the spg1n-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.

156 In vitro function of the aryl hydrocarbon receptor predictor 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 evoke adverse effects on embryos have been observed among oviparous 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 of these compounds in vertebrates. Biologically meaningful endpoints in the aquatic environment are linked to two AHRs, an AHR1 and an AHR2. Prior investigations demonstrated that sensitivity to activation of the AHR1 (EC50), but not AHR2, in an in vitro luciferase reporter gene (LGR) assay using transfected COS-7 cells was predictive of the sensitivity of embryos (LD50) 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 predicts sensitivity to DLCs of embryos of the two 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 vivo sensitivity to activation (EC50) of the model DLC 2,3,7,8-TCDD of AHR1 and AHR2 among 8 species of fish known sensitivity of embryos to TCDD. There was no statistically

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significant linear relationship between in vitro sensitivity of AHR1 and in vivo sensitivity among the investigated species (R²=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 (R²=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 (R²=0.87, p=0.0001). It was suggested that this linear relationship can be used to predict the in vivo sensitivity of any species of oviparous vertebrate to any DLC for application to ecologic risk assessment.

157 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 project, we established the three analytic liver cell lines eWoCl3, eWoCl4, and eWoCl5 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 (H4IEE) and fish (ZFL), respectively. With these cell lines bioassays for CYP450 activity and induction were conducted. The assays included enzymes like 7-ethoxyresorufin-O-deethylase (EROD), 7-pentoxyresorufin-O-dealkylation (PROD) and 7-benzoxyresorufin-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.

158 In vitro hepatic and gastrointestinal 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 and the environment can be significantly altered. In vitro bioassays, 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 evaluated in vitro hepatic and gastrointestinal biotransformation rates for chemicals (log Kow >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. As a result, 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

159 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 bandgap 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 MnO. NPs as models for dissolution and bandgap paradigms, respectively. CeO2 NPs were also assessed to consider the potential ability of producing ROS via Fenton like reactions in vivo. The hypotheses were tested by exposing for 24 hrs eyster 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 parameter. This work provide 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 properties. 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 MnO2 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.

160 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 automotive 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 findings provides a new safety information for automotive industry using nanoscale transparent Cu Phthalocyanine for advanced coatings.

161 Challenging sea urchin defense with unminated 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 annotated (PSNH) 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 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 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 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 NPs on ATP-binding cassette (ABC) transporters was probed with the calcine-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 ABCG1 blocker MK-571 was added. Overall, our data suggest that PSNH 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.

162 TBD

163 Poster spotlight: TU071, TU072, TU073, TU074

_Aquatic and Terrestrial Plant State-of-the-art Research linking ecotoxicology and exposure of chemicals_

164 Effects of the herbicide metalufoxuron-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 metalufoxuron-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, 0.0388 (EC50 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, Kruskal Wallis test) with an ED50 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. tanacetifolium_ and _C. cyamus_) 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. cyamus_ and _P. tanacetifolium_ 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.

165 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 of the threshold value and of the considered technical data. The impact of the threshold value is more pronounced over small changes and was considered to be critical. Conclusions are based on the absence of 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 which perform at 95% confidence. A simplified approach would increase buffer zones 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”_

166 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 reduct-oxidative 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. 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, metallothionein 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 potentially useful in environmental monitoring. In the present study, we investigate seed germination parameters, oxidative status and gene expression level of genes involved in metal detoxification 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 (CuSO4, 5HO, 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 PathMT gene expression were significant. Highest cupric ions amount maintained reduct balance heavier in their oxidative state and also regulated the expression of their own _CytP450_ 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
167 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" (Malbty et al., 2010), which took place in January 2008 in Wageningen, the risk assessment of aquatic macrophytes was discussed. This resulted in an investigation of the protective setup 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 importance of 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..._SETAC Europe 27th Annual Meeting Abstract Book_
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 typical growth patterns of this species in natural aquatic ecosystems and the bioaccumulation potential of polycyclic aromatic hydrocarbons (PAHs) present in these ecosystems. If the relevant information on these topics is available it can be used to improve already existing mechanistic effect models. Having fully validated effect 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.

168 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 hydrological characteristics of a particular site with respect to the specific compounds and conditions into account. The standard approach at the European level predicts surface water concentrations of a pesticide for an entire site 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 on 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 approaches offer several advantages such as the possibility to assess numerous weighted average (TWA) concentrations instead of the maximum. However, 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 on 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 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 in 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)**

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

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Release of anthropogenic carbon dioxide in to the atmosphere and subsequent absorption by surface waters is resulting in increased pCO2 in aquatic environments. Furthermore, active pharmaceutical ingredients (APIs) are increasingly being found in sewage effluents and receiving waters, primarily as a result of human use. Traditionally, these have been studied in isolation, although factors such as pH and pCO2 can alter ionisation and bioaccumulation potentials of many APIs, altering their toxicity. This study aims to investigate the hypothesis that changes in pCO2 alter the uptake and effects of common chemical contaminants. We used the ragworm *Helistoe 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 pCO2 and having drug targets that are conserved in marine invertebrates. Exposures were performed under pCO2 conditions equating to pH 8.10, 7.75, 7.40 (pH 7.40), 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 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 increased pCO2 concentrations may impact the toxicity of contaminants, in particular APIs.

170 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 interactions of OA with 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 pCO2 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 pCO2 levels. For example, in the mussel *Mytilus edulis* copper-induced DNA damage was 90% greater when exposed under OA conditions (1373 μatm pCO2) compared to ambient conditions (436-4 μatm pCO2). 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.

171 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 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 copepod species *Calanus glacialis* and *C. finmarchicus* to pyrene at three temperatures: 2, 6 and 10°C. Both species coexist in the Disko Bay, Greenland. However, *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 AR6. Both species coexist in the Disko Bay, Greenland. 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.

172 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 draygonfly 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 arabis) 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 ImageJ. There was no effect on 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±5.3, 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. arabis tadpoles to predator kairomones are dose-dependent, but follow an additive mode of action.

173 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 (C6H5Cl2O2S) 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 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. However, 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 (chlopyrifos) 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 the immune system with dramatic decrease of phagocytic efficacy 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

174 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 in 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 (θTWA) of eleven PAHs, one nitro-PAH, and four oxo-PAH derivatives natively present in a fuel oil soot from commercial boat fueling. Using an in vitro-three-stage gastrointestinal model. We then calculated a limiting bioaccessibility (θlim). 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. Silicon absorption was expected to promote desorption by sterically crowding the concentration across the soot. The calculated θTWA and θlim included the sum of fluid-phase and silicone-phase chemical. As predicted, silicone significantly increased θTWA by reducing the labile fraction remaining on the soot residue after digestion. The θlim was independent of gastric pH and added dietary proteins and carbohydrates, but increased with bile acids concentration, significantly increasing at the physiological pH (5.0 – 7.5), 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 θlim ranged from ~30 to ~65% among the PAHs and PAH derivatives, which were surprisingly high. The θlim was not correlated with molecular size or Koc, 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 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.

175 Application of an antibody-based bioassay 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 30 minutes of collection. A bioassay was developed 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.

176 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 scale 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 methods have the advantages of being 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-experimental setup was designed. Two different experimental setups 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]. Further, two different concentrations were used to determine 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 experiments, PAs with a log10Kow<5.5 have already reached equilibrium. For the fully in situ situation only PAs with log10Kow<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.

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

Analytical tools for the holistic assessment 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 bacteria that is susceptible to the chemical tested is called minimum inhibitory concentration (MIC). The objective of the present work was to provide a series of analytical tools (validated quantitative and sensitive biochemical methods) to assess the ambient concentrations of selected antibiotics, including surface water, groundwater, marine coastal water, sediment and biota. The data obtained allowed assessing their occurrence and concentration of the antibiotic resistance 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, 1992-2002: a national reconnaissance. Environ Sci Technol, 36:1202-1211. [2] Berkner S, Konrad S, Schöf Feld J. 2014. Antibiotic resistance and the environment—there and back again. EMBO rep 15:740-744 [3] Martinez 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’).

Low-energy Downflow Hanging Sponge (DHS) bio-reactors reduce antibiotic resistance genes for decentralised wastewater treatment applications

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Billion-pound worldwide lead to inadequate 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

177 How to consider bioavailability in the assessment of construction products


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-toxicological testing provides information on the environmental risk without the need for 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 allows 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.

178 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 persistence of NER may be associated with 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. 14C) and cannot be measured with conventional chemical analyses. Regulations ask for understandable and practical approaches to be taken. The approach chosen by Calvo et al. (1) has been followed and considered are: the water phase, in which concentrations/activity can be measured using passive sampling or extractions with 0.01 CaCl2 (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 14C-labelled compounds. With 14C-experiments radio activity is measured but the substance identity remains unknown. The distribution in the soil of 14C was followed for 6 months. Toxicity was tested in both freshly spiked and aged soils. TNT, Cypermethrin and Carbendazin were used as test chemicals and results will be presented. For 14C-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 retardation effect 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 bacteria that is susceptible to the chemical tested is called minimum inhibitory concentration (MIC). The objective of the present work was to provide a series of analytical tools (validated quantitative and sensitive biochemical methods) to assess the ambient concentrations of selected antibiotics, including surface water, groundwater, marine coastal water, sediment and biota. The data obtained allowed assessing their occurrence and concentration of the antibiotic resistance 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, 1992-2002: a national reconnaissance. Environ Sci Technol, 36:1202-1211. [2] Berkner S, Konrad S, Schöf Feld J. 2014. Antibiotic resistance and the environment—there and back again. EMBO rep 15:740-744 [3] Martinez 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’).

180 Low-energy Downflow Hanging Sponge (DHS) bio-reactors reduce antibiotic resistance genes for decentralised wastewater treatment applications

J. R. Newcastle University / School of Civil Engineering and Geosciences; L. Su, Chinese Academy of Sciences / Institute of Urban Environment; C. Harwood, Newcastle University / Institute for cell and molecular biosciences; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; Y. Zhu, Chinese Academy of Sciences / Institute of Urban Environment; D.W. Graham, Newcastle University / School of Civil Engineering and Geosciences

Billion-pound worldwide lead to inadequate 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 existing peri-urban communities. Recent work has shown Downflow Hanging Sponge (DHS) bio-reactors 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 anaerobic conditions, creating sequential aerobic-anaerobic 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 influent and effluent and influent influent and effluent 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. Our results are very promising for prospectively reducing ARG loads in domestic wastewater and should be promoted for decentralised use in the emerging world.

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 μg/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 cytophactamers 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 taxon 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 ecotoxicity endpoints and AMR development. Our key finding are: 1) the sensitivity range for cytophactamers exceeds the range over twofold higher than their use in agriculture results in flow of antibiotics and antibiotic resistant organisms into the natural environment. 2) Environmental concentrations of antibiotics are very low (mostly in the ng/L range[30]), recent studies have shown that these may exert enough selective pressure to select for antibiotic resistance[15, 16]. Currently there are no standardised ecotoxicological assays which assess a compound’s potential to select for antibiotic resistance, methods used to determine minimal selective concentrations (MSCs’ - the lowest antibiotic concentration which selects for resistance[15]) in other studies[44, 45] 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 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 relevant concentrations, and that growth of a natural complex community may be useful in determining MSCs. [1] O’Neill, 2014. O’Neill Report Wellcome Trust. Review on Antimicrobial Resistance. [2] O’Neill, 2014. O’Neill Report Wellcome Trust. Review on Antimicrobial Resistance. [3] O’Neill, 2014. O’Neill Report Wellcome Trust. Review on Antimicrobial Resistance.

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

183

Poster spotlight: TU141, TU142, TU143, TU144

184 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 Nafón 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. Families 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 ZPr00-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 biogemoter 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.

185 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 bisides 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 (haphtophyte) 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 0
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 EC50 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 antagonism. Single exposure results on *Tisochrysis* *lutea* showed a very high toxicity of irgarol, diuron and ZnPtwith EC50 values below or in the µg 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. Within the binary mixtures, however, changes 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 statute 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.

186 Sensitivity of Caenorhabditis elegans to mixtures of zinc, copper and cadmium s. mosson, University of Antwerp; G. Baggerman, Flemish Institute for Technological Research VITO / Department of Biology SPHERE; R. Blust, University of Antwerp; S. Husson, University of Antwerp / Biology

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 main challenges in ecotoxicology is to obtain insights in mixture toxicity 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), 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.

187 Effect of temperature on chronic Ni toxicity to *Daphnia magna* along four generations C.S. Pereira, Ghent University; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; R. Blust, University of Antwerp; K. De Schampaere, Université Catholique de Louvain / Applied Ecology and Environmental Risk Evaluation

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 (<24 h) collected from the 3rd brood of at least three 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 exposed to the 5th brood (EC10) for Ni increased along generations. However, for FO the EC10 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.

188 Does prior exposure to heavy metals protect future generations of plants to metal stress? E. Saenen, Belgian Nuclear Research Centre (SCK-CEN); E. Lauwers, R. Nauts, M. Van Hees, A. Van Gompel, J. Wannijn, N. Horremans, SCKCEN

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 against these changes. 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 CHD3 of the U-seeds as compared to 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 D. Spurgeon, Centre for Ecology & Hydrology; H. Hesketh, M. Heard, Centre for Ecology and Hydrology; E. Lahive, Centre for Ecology & Hydrology (NERC); C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology; A. Robinson, Centre for Ecology and Hydrology; A. Rortais, EFSA European Food Safety Authority; J. Done, European Food Safety Authority EFSA / Scientific Committee and Emerging Risks Unit Department of Risk Assessment; A.A. Horton, Centre for Ecology and Hydrology

For laboratory testing, assessments involving bees generally start with acute studies for solitary bees, honeybee and bumblebees: differential sensitivity and mixture interactions? These trials were conducted used a tailor-made design based on relevant publications and consultations with representatives of a social bumble bee species (*Bombus terrestris*) and 8 semi-field tests were done with *Osmia bicornis*, a solitary bee. *Apis mellifera*, further, only limited information on mixture effects on bees is available. We have developed and trialled toxicity tests that allow for exposure of honeybees for three treatments: *A. mellifera* and *O.* *bicornis*, bumblebees *Bombus terrestris* and a solitary bee *Osmia bicolor*. 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 derivate and used to fit dynamic energy budget toxicity (DEBbox) 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 growth rates. 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.

190 Interspecific sensitivity of wild bee species and the relevance of the honey bee as a sole test organism for the risk assessment of pesticides. A. Rortais, EFSA European Food Safety Authority EFSA / Scientific Committee; P. Uhl, L.A. Franke, University of Koblenz Landau / Institute for Environmental Sciences; L. Jeker, Agroscope; C. Rehberg, N. Schnetzer, R.S. Schulz, University of Koblenz Landau / Institute for Environmental Sciences; P. Stahlcimcut, University Koblenz-Landau / Institute for Environmental Sciences; C. Wollmann, University of Koblenz Landau / Institute for Environmental Sciences; C. Bruehl,
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 EFSAs a proposed an additional assessment factor of 10 to account for interspecific differences in bee sensitivity. Furthermore, the semi-field to include one bumblebee Bumblebus terrestres in and the red mason bee Osmia bicornis into risk assessment. Data from 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 appropriate. We performed multiple dose-response contact toxicity lab tests with wild bee using dimethoate as a toxic standard. Afterwards, we generated a species sensitivity distribution (SSD), derived a hazardous dose (HDS) and its lower 95% confidence limit (lower limit HDS). Furthermore, we determined the acute toxicity of multiple insecticides towards the proposed test species O. bicornis and compared it to honey bee data. HDS was calculated to be 0.08 µg a.i./bee and the lower limit HDS 0.04 µg a.i./bee. To include limits for bumblebee, LD50 for A. mellifera calculated from literature data divided by a safety factor of 10. B. terrestres 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. terrestres 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 organ in pollinator risk assessment lower tier testing.

191 Summary of an ICPRR Non-Apis Workshop - Subgroup Higher Tier (Bumblebees and Solitary Bees) with recommendations for a semi-field experimental design

S. Kimmel, Innovative Environmental Services (IES) Ltd. / Ecotoxicology; S. Morgen, Testapi; B. Peters, Tier3 Solutions GmbH; C. Schneider, BASF SE / Ecotoxicology

The publication of the proposed EFSA risk assessment guideline 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 setup. The ringtest design was based on the EFSA guidance document, ÖEPP/EPPO Guideline No. 170 and results of discussions regarding testing solitary bees during the meetings of the ICPRR non-Apis working group in 2015 and 2016. Ringtests were conducted with two different test organisms, one representative of a social bumble bee species (Bummbus terrestres 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 9 different species including the two semi-field test organisms. Two treatment groups were always included in the ringtest: an untreated control and dimethoate as a toxic reference item (opportunistic i.e. brood affecting substances (tenoxycarb)). 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 Day 50 was 0.02 µg a.i./bee. The lower limit HDS is equal to the mean 48h LD50 for A. mellifera calculated from literature data divided by a safety factor of 10. B. terrestres 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. terrestres 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 organ in pollinator risk assessment lower tier testing.

192 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. Some bee species 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 using 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 field study 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 coconet/egg 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 CGA32704. 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.

193 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 rapedeseed Brassica napus inflorescences and their respective pollinator foods (pollen, nectar and honey). The 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 bee solitary 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 analysis. The water content was used to calculate the dry matter content. The dry matter content was used for 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)

194 Linking environmental water requirements to freshwater biodiversity damage at multiple scales

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Freshwater ecosystems are affected by 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

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environmental relevance of water stress indicators (e.g. AWRRe). 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 multiple 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.

195 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 database). 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 species distribution 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 of habitat alteration. This characterization 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.

196 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 resulted in a new 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 traditional 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 model changes that have recently been proposed to ECHA by the Dutch National Institute for Public Health and the Environment (NUTRIM/SST) and the Dutch Environment Agency. 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 philosophies behind.

197 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 (Vwua) and degradative water use (Vwau). Vwua equals the volumetric change between water extraction and wastewater effluent. Vwau for emissions i is defined by the emission load and the reference emission concentration in a water quality standard. Vwua and Vwau 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 washing/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 0. For every unit factor in the indicator score the following 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 WSAF 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.

198 Poster spotlight: TU208, TU209, TU210, TU211
Looking across organizational boundaries: exchanging ideas on mechanistic modelling between SETAC and the International Water Association (IWA) (I)

199 Overview of major topics from SETAC & IWA L. Zeng, Ghent University; K. Villee, Eawag - Swiss Federal Institute of Aquatic Science and Technology; E. Zimmer, IBACON GmbH; T. Gouin, Unilever / Safety and Environmental Assurance Centre
200 Developments in Integrated Urban Drainage
L. Vezzaro, DTU (Technical University of Denmark); W. Rauch, University of Innsbruck

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

202 Developments in Drinking Water Treatment
B. Martig, 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.

203 Discussion

204 State-of-the-art tools for exposure and chemical risk assessment
D. Van de Meent, Independent Consultant; J. Strujs, Radboud University Nijmegen; D. De Zwart, DiZ 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 ‘simplebox/treat’, 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). An This presentation illustrates the use of the REACH models SimpleTreat and SimpleBox, as combined in UBA’s ‘simplebox/treat’ 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).

205 Mechanistic effect models for ecological risk assessment: where next?
V. Ducrot, Bayer CropScience AG / Environmental Safety Ecotoxicology; P. Thorbek, Syngenta / Environmental 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 assessment as well as method to address the model uncertainty. Notifiers may 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.

206 Discussion

207 Integrating treatment facility and river network information to model spatially-explicit environmental concentrations of down-the-drain substances: iSTREEM
C.M. Holmer, R. Vanshri, Waterborne Environmental, Inc.; P. DeLeo, A. 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 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.

208 Cost-Effective and Integrated Optimization of the Urban Wastewater System Einzelheiten
L. Benedetti, Waterways; T. Flameling, Waterschap De Donk; J. De Klein, Wageningen University / Aquatic Ecology and Water Quality Management; J. Langeveld, PartnersUrbanWater; i. nopens, Ghent University; S. Weijers, Ghent University / BIOMATH

The Donmel 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 Donk, 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 toolbox with which all systems in the city are connected. The model is then implemented 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 IWS 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.

209 Discussion

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

210 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 Kow). 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 Kow = 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 m/l/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 Kow values (>9) were closer in agreement with in vivo BCFs when the correction factor f0 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 at avoid toxic screening concentrations due to classical log Kow-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.

211 Screening for low aquatic bioaccumulation: prediction potential for replacing BCF tests based on physicochemical properties M. Nenzia, Analytisches Laboratorium; R. Kühne, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; A. Lombardo, RBCCS - Istituto di Ricerche Farmacologiche Mario Negri; S. Strempel, ETH Zurich; G. Schuermann, 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 as 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 organonemethyl, 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 lower B or (very) similar (BCF). Benchmarking study to support regulatory acceptance of the application of in vitro-in vivo extrapolation to predict bioaccumulation potential of chemicals (EFSA 2014). The prediction 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 bioaccumulation testing of 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.

212 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, Rossoum, 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.

213 The Effluent Toxicity Assessment Toolbox - International Perspective on Tools and Concepts and Opportunities for Animal Alternatives T. J. Gartung, King, U.S. EPA / National Aquatic Test Guideline Ecosystem Division; M.R. Embry, ILSI Health & Environmental Sciences Institute (HESI); S.E. Belanger, The Procter & Gamble Company / Central Product Safety Dept; T. Braunbeck, University of Heidelberg / Centre for Organisnal Studies; J. Butler, ExxonMobil Biomedical Sciences, Inc / Environmental Toxicology and Chemistry Laboratory; P. Doran, E.R. Dorn Associates, LLC / Fish and Wildlife Health Research Institute for the Environment and Climate Change Canada or the US Environmental Protection Agency; P.M. Wilson, Sanofi U.S., Inc. / Health, Safety and Environmental; G. Whale, Shell Health - Environmental; M.K. Sellin Jeffries, Texas Christian University / Department of Biology; R. Journel, SANOFI; M. Leonard, lOREAL SA; M.E. McMaster, Environment Canada / Ecotoxicology and Wildlife Health Assessment Centre; S.A. Hughes, Shell Health - Americas / Shell Health Risk Science Team;"
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 have been tested in a variety of industries. The indirect effect of metal toxicity 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-ethinylestradiol (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-ethinylestradiol (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 being, however, the measurement group is not 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.

217 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, we hatched zebrafish and also video taping 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 (LC50 Cu 135.6 μg/L, LC50 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 (EC50 Cu 34.46 μg/L, EC50 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.

218 Effects of environmental pollutant mixtures on immune function and disease susceptibility in wildlife
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Depicted in aquatic environments, while non-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 studies. In one study, we used real environmental exposure 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 truncatus), harp seals (Pagophilus groenlandicus), and hooded seals (Cystophora cristata). Secondly, we investigated the effects of particulate cocktails on disease outcomes using influenza A infections in Mudin-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.

219 UK MSFD assessment: spatial and temporal analysis of biliary 1-hydroxyphene, ethoxresorufin-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 samples 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 Framework (CSEM) data has been collected for EROD activity, biliary PAH metabolites and AChE activity in flatfish (Dab Limanda limanda, plaice Pleuroneustus 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 breaches of the BAC, 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

220 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 waste disposals and deposition. Their 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 form of ENMs whereas the dissolved form 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 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 EC90, 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 functions. Further ecosystems functional diversity and soil quality will be affected by addition of ENMs. These results are essential to understand in pursuing a naturally realistic assessment of environmental risks of ENMs.

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

M. McKee, J. Koester, University of Bremen / Center for Environmental Research and Sustainable Technology; J. Filser, University of Bremen / Center of Environmental Research and Sustainable Technology

The use and application of silver nanoparticles (AgNP) has increased in recent years, in line with the related environmental concern. Several 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 ion exchange capacity 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, and also to better understand the mode of action and the impact of the soil matrix on the toxicity of AgNP in soil. Collembola are important contributors in the biodiversity of soil ecosystems. 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 peak 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 this in our next experiment and analyse the respective 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.

222 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 nanotechnology 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 WC00 and CuO NMs on survival, reproduction, and gene expression for four consecutive generations, plus an additional two generations in clean soil to study recovery. F. candida were not shown and the study investigated the effects 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 NMIs 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 Is were two-fold to three-fold more toxic in the 3rd 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 NMIs, 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 NMIs on soil invertebrates.

223 Copper toxicity in Collembola: a comparison between nano and non-nano nanoparticles J.T. Neves, Department of Biology & CESAM - University of Aveiro / Biology; D. Nunes Cardoso, CESAM, University of Aveiro / Department of Biology and CESAM; C. Malheiro, Department of Biology, University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology & CESAM, 3810-193 Aveiro, F.J. Young, University of Calgary / Department of Biological Science; S. Loureiro, Universidade de Aveiro / Biology

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 pesticides, however, are capable of interacting with their targets in biological systems due to their extensive use and applicability. The objective of this work was to compare the toxicity of a copper nanoparticle (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 run in two soils, Lafa 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 nanoparticle Kocide 3000 shows a similar toxicity trend when compared to the correspondent non-nanoparticle, 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 Lafa 2.1, probably due to a lower fitness performance of collembola related to the higher availability of Cu2+ 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

224 Do we worry too much? Nanoparticle and other stressors' effects to a soil-grown legume at realistic exposure levels E. Schwab, CEREGE / Civil & Environmental Engineering - CEINT; L. Pagano, University of Palermo / Chemical Sciences; D. Borschneck, C. Levard, B. Angeletti, CEREGE; J.C. White, Connecticut Agricultural Experiment Station / Department of Analytical Chemistry; J. Rose, CEREGE; M. Auffan, CEREGE / International Consortium for the Environmental Implications of Nanotechnology

Engineered nanomaterials (ENMs) are relatively novel contaminants of emerging concern concerning the environment soil and human health. ENMs are small in size (≤100 nm), and can have unique properties different from the parent material. This makes them potentially hazardous to soil organisms. In this work, we investigated the effects of ENMs on the growth and development of a soil-grown legume (Pisum sativum). The legumes were exposed to different concentrations of nanoparticles and conventional pollutants under field conditions. The results showed that ENMs, particularly AgNPs, significantly reduced the growth and development of the legumes. However, the effects were less pronounced in the presence of conventional pollutants. These findings highlight the importance of considering ENMs as a potential environmental risk and the need for further research to understand their long-term effects on soil ecosystems.

225 Pesticide toxicity is enhanced by pre-exposure to wastewater J. Zahrend, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; D. Englert, Institute for Environmental Sciences / Institute for Environmental Sciences; S. Poganiuch, University of Koblenz-Landau / Institute for Environmental Sciences; S. Lüderwald, Universität Koblenz-Landau / Institute for Environmental Sciences; T. Schulze, Helmholz centre for environmental research - UFZ / Institute for Environmental Sciences; M. Bündschuh, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Swedish University of Agricultural Sciences

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 streamwater, WW, or a combination of both, with or without a micronutrient 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 excessive stress could 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 assembles’ long-term adaptational mechanisms to WW exposure and their consequences in a "multiple stress" environment to ensure that functional integrity is safeguarded.

227 Effects of fungicide exposure and feeding pressure on aquatic fungi across different biogeographical regions
V.C. Schreiner, University of Koblenz-Landau; A. Feckler, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; D. Fernández, University Koblenz-Landau / Quantitative Landscape Ecology; K. Frisch, University of Koblenz-Landau; M. Bundschat, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; J. Rasmussen, Aarhus University / Institute of Environmental Sciences and Assessment; B.J. Keftord, University of Cambridge / Institute for Applied Ecology; R. Schaefer, University Koblenz-Landau / Institute for Environmental Sciences 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 fungicide colomisation cycles in Germany, Sweden, Denmark and Australia. After colonising leaves bags of the target or non-target fungus species, we considered 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: 2TU-3, 2TU-2, 2TU-1), with a two-day peak, followed by a 12-day base. For each concentration, the experiment was run with and without local shredders (gammaries 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 assessed: OMD, fungal community structure and fungal biomass. OMD strongly differed among the countries in which the experiment was conducted (four factorial ANOVA).

228 Effects of fungicides and nutrients on heterotrophic microbial communities under global change projections - the importance of exposure history
A. Feckler, Swedish University of Agricultural Sciences (SLU) / Department of Aquatic Sciences and Assessment; W. Goedkoop, Swedish University of Agri Sciences / Department of Aquatic Sciences and Assessment; R. Bundschat, SETAC Europe Office / Institute for Environmental Sciences; K. Kengott, University of Koblenz-Landau / Institute for Environmental Sciences; M. Konschak, University Koblenz-Landau / Institute for Environmental Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences; J. Zubrod, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; M. Bundschat, Department of Aquatic Sciences and Assessment Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment. 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 NO3-N/L) on the structural and functional plasticity of leaf-associated microbial communities with differing exposure histories (pristine vs. agriculture) in a 2x4x4 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<0.013). Higher fungicide exposure 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 prey/predator-induced eicosanoid response in the aquatic 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-regulation of chemotrophic enzymes 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 generally under pressure by a agricultural expansion, results point to a broad scale loss of biodiversity and ecosystem functions at the base of food webs that ultimately jeopardizes the integrity of stream ecosystems.

229 CASCADING AGRICULTURAL CONSTRAINTS ON PLANT LITTER FUELED TROPHIC LINKAGES
E. Hunting, CML Leiden University; H. Barmentlo, M. Schrama, CML Leiden University / Conservation Biology
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. We show that 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-biode 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 alter 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.

230 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 and biotechnologies. Several phytotechnologies can be applied to produce usable 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 the groundwater and aerial parts and preventing their bioaccumulation in the food chain as well as dispersion by natural agents (2) phytoremediation, 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, the former poplar planting was fouled by removing ca. 50% of Fe and Mn from contaminated biomasses, while Ecocatalysis was also successfully applied to i.e. Zn hyperaccumulating species (A. halleri).

231 From bioavailability science to bioremediation of PAHs: where are the limits for risk reduction?
J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo; R. Posada, IRNAS CSIC / Agroquimica y Conservacion del Suelo; J. Garcia, Instituto de Recursos Naturales y Agrobiologia de Sevilla CSIC; M. Cantos, IRNAS-CSIC / Spanish National Research Council 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 to be a measure of the attainment of bioaccessability (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 related to the analysis of the processes that control bioavailability under environmental samples, and it has related new strategies to improve bioavailability (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: 776-777, 2015).

234 Recycling of waste water treatment sludge as a substitute soil amendment for mine waste reclamation
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The last two fluorosphars mines in France, closed in 2006, were opencast sites (Montroc and Le Moulin) 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 Moulinial 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: grasshouse trials using Lotus corniculatus and Festuca arundinacea for productivity tests, and the Rhizotest root mat technique (ISO 16198) to assess the phytovailability 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 toxic effects on metals. The encouraging results provided the basis for field trials at the two sites (Mont-Roc and Le Moulinial) 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
235 Updates to the proposed Canadian regulatory framework for the environmental assessment of new active ingredients in human and veterinary drugs
A Canadian regulatory framework has been developed specifically for active ingredients in human and veterinary drugs 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 medicines.
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.

236 Estimating soil emissions and toxicity impacts from the application of livestock manure: application to heavy metals at national scale
A. Leclerc; DTU / Management Engineering; A. Laurent, DTU / Division for Quantitative Sustainability Assessment DTU Management Engineering

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 may impact the environment. The 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, and 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 metal concentration ratios used in aquatic chronic metal mixture studies often investigate mixture toxicity at equitoxic concentrations (PECs). The 2008 guidelines specifically recommend the use of standard regional conditions 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.

237 Challenges in exposure modelling of fish veterinary medicines
F. Ercher; Cambridge Environmental Assessments; J. Carnall, Cambridge Environmental Assessments CEA

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) 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 specifically recommend the use of the models designed by the Scottish Environmental Protection Agency (SEPA) for the regulation of the fish farming industry. In the context of EU regulations, the acute risk associated with use of the aquaculture veterinary medicinal product is evaluated based on Tier 1, Tier 2 and Tier 3 market approach. In order to fit 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 to which combination of farm parameters and environmental conditions can be considered a realistic worst case.

References:

238 A new approach for supporting the comparison of the benefits and environmental risks of veterinary medicinal products
J. Chapman, Environmental Department University of York; A. Boxall, University of York / Environment Department; L. Porsch, R. Vidaurre, Ecologic Institute; T. Backhaus, University of Gothenburg / Department of Biology and Environmental Sciences; C. Starr, Starr Environmental Consulting; A. Boxall, University of York; University of Gothenburg; J. Chapman, Environmental Department University of York; G. Jones, Fera Science Ltd; P. Howley, University of York

Vernacular 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 comparing approaches for risk-based 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 for further research regarding the application of risk-benefit assessments on the farm scale.

239 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 applied systematically 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 PHR 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 (v) the resulting need to compare both environmental and toxicological properties.

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

240 Chronic metal mixture toxicity to freshwater organisms: does the metal concentration ratio matter?
C. Ngu, University of Ghent / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; T. Van Regenmortel, Ghent University / GhEnToxLab; D. Deryuyter, University Ghent / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; K. De Schamphelaere, Ghent University (UGent) / Applied Ecology and Environmental Biology Metal mixture studies often investigate mixture toxicity at equitoxic combinations (i.e. equal toxic unit for each metal mixture which must 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 conditions...
concentration ratios) and environmental (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 chemicals and metals. 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 experiments were always antagonistic relative to the concentration addition model, while mostly non-interactive in environmental. This suggests that chronic metal mixture effects observed in equitoxic experimental designs can not be simply extrapolated to environmentally realistic concentration 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 scenarios. Our study highlights that metal concentration ratios need to be carefully chosen to increase the environmental relevance of chronic metal mixture studies.

241 Context Matters: What Is Known about the Additivity of Toxicity in Ni-Containing Metal Mixtures

J.S. Reuven, Amplex Limnology Professionals LLC; E.R. Garman, NIPERA / Ecotoxicologist; C.E. Schlekat, NIPERA

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 equitoxic combinations. 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 5 metals combined with Ni. A large majority (62%) of the combinations of publication and water type used some form of actual or reconstructed 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 exposure data, which is still the case in some current metal-mixture toxicity publications.

242 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 land 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 evaluate 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. This involved toxicity data on soil activity (through bait-lamina), macrofauna 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 meso fauna, especially on enchytraeids and collemobans. 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.

243 Understanding the effects of complex (and realistic) metal mixtures to soil invertebrates

J. Renaud, CFE Centre for Functional Ecology; M. Cousins, University of Saskatchewan; K. Jegede, University of Saskatchewan / Toxicology Centre; B.A. Hale, University of Guelph / School of Environmental Sciences; J.F. Sousa, University of Coimbra / Department of Life Sciences of University of Coimbra; S. Siciliano, University of Saskatchewan / Department of Soil Science

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 many sediments. As a result metals are persistently widespread and at high concentrations in the environment. The toxic impacts of metals generally on metals 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 ternary 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 Ophiura ninitens), using a fixed ratio array design. Fixed ratio designs use a constant ratio of different metals across increasing doses and the fixed mixture ratios 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 Ophiura ninitens 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.

244 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 aquatic toxicity 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 aquatic toxicity of U. Cd and a mixture of both metals to the freshwater macrophyte Lemna minor L. Therefore, the accumulation and toxicity to U in the presence of varying proton or cation concentrations (Ca²⁺, Mg²⁺, Na⁺, K⁺) was investigated for Lemna minor L. growth inhibition test. From the experimental data complemented with speciation calculations (WHAM7) A Biotic Ligand Model (BLM) for U in Lemna minor L was set up. In addition possible interactions between U and Cd were tested in a second 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 for U and Cd was fitted for U, however, it was shown that the 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 experimental results of all three models. It is shown that the two-site model provided the best fit, despite the fact that the corresponding accumulation model
Advancements in life cycle impact assessment and footprint method development (II)

245 Evaluation of Product Impacts on Biodiversity: The Product Biodiversity Footprint project
C. Catalan, I Care & consult; A. Asselin, Consultant; S. Rabaud, H. Anest, B. Leveque, G. Neveux, I Care & consult
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, emissions, pollution, 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 dynamical fields. The objective of this 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.

246 Forest Product LCA: Carbon Form, Fire, Fuel and Fate Rules
M. Vlieg, The Evah Institute / 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 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, emissions, pollution, 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 dynamical fields. The objective of this 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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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.

248 Market prices based valuation of abiotic resources in environmental assessment
S. Standaert, T. Huppertz, E. van Overbeeke, 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 for price of monetising non-renewable resource consumption?”. Based on an analysis of the market failures affecting the market price, we observe the deviation of the main identifiable bias among 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 retroputed 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 correction. The method leads to proposals of monetisation factors and characterisation factors for non-renewable resource consumption.

249 Poster spotlight: TU212, TU213, TU214, TU215

Bridging between ecology, ecotoxicology and ecosystems services (I)
Looking across organizational boundaries: exploring ideas on mechanistic modelling between SETAC and the International Water Association (IWA) (II)

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

A. Vacek, Alterra Wageningen University and Research Centre / Agricultural and Environmental Risk Assessment Team; H. Baveco, Wageningen Environmental Research; C. Lindin, 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 source-sink 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

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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 km². 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.

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

257 ChimERA: integrating fate and effect modelling
K. Viana, 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.

258 Discussion

259 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 module), new models for MP phases are needed. Integrated exposure assessment based on MP modelling should reflect the remaining part 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 developed, with 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 managers 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.

260 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, gaia - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change; T. Strauss, Research Institute gaia / gaia - 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 imminent 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-Carlo 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 scenerios 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.

261 Discussion

262 Communication Approaches to implement mechanistic modeling in decision making
F. Verdouw, 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 Bionet, 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. Bionet 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 design allows for easier modelling of it. The bottom-up and bottom-up structured organisation of model components is difficult as interpreted by the option set. However, 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.

263 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 evaluation. Additionally, Sentinel units 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 higher-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 used, 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 responses after exposure conditions. Currently, no model as been agreed and validated at EU levels. TK/TD models could become a very useful tool for risk assessors in the future, but solutions have to be found. A tool box, developed within academics, industry and regulators could be developed and widely used in a harmonised way for active substance monographs and/or for registration of plant protection products.

264 Discussion and Forging Collaboration

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

265 How to use Fish Embryo Acute Toxicity (FET) Test Guideline (OECD 236) to fulfill the information requirements of REACH Regulation

A. Nyman, European Chemicals Agency (ECHA); M. Sobanska, European Chemical Agency; R. Casamenti, S. Gutierrez Alonso, W. De Coen, European Chemical 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.

266 Benefits of Using Read-Across and In Silico Techniques to Fulfill Hazard Data Requirements for Chemical Categories

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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 (QSAR) 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.

267 The Environmental Read-Across Assessment Framework for environmental hazard and fate endpoints

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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. Substances in categories 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 very useful for all readers. RAAF 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 underpinning 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.

268 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-TTC’s summarize the wealth of ecotoxicoological information as Predicted No-Oberved Effect Concentrations (PNECs) on diverse chemical substances in the form of statistical databases. It is therefore possible to predict the relative reliability of entity-level approaches by using experts in the field to source data and bridge the data that 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 organisms 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. For example, the datasets 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.

269 ecodatabank - Big data techniques to create trusted, actionable ecotox data A. Beasley, The Dow Chemical Company / TERC; M.R. Emery, ILSI Health & Environmental Sciences Institute (HESI); R.R. Otter, Middle Tennessee State University / Biology; K. Allen, Kramer Allen Consulting; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; A. Lillcrap, NIVA / Ecotoxicology and Risk Assessment; D. Major, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; T.W. Valenti, Syngenta Crop Protection LLC / Environmental Safety 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-disciplinary database that is 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 ecodatabank 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 ecodatabank interface will be free and publicly available so that users can easily interact with datasets and streams. The ecodatabank 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

270 Arsenic uptake by rice: Interactions with Silicon M. Limmer, A. Seyfert, University of Delaware / Plant and Soil Sciences Rice (Oryza sativa) is a globally important food crop that is frequently contaminated by arsenic. Research has shown that silicon is an effective additive to minimize uptake of arsenic, 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 the toxicity of DMA, where higher 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.

271 Do fatty acids and methylmercury impact the function of adipose tissue in rainbow trout (Oncorhynchus mykiss)? G. Tintant, Université Catholique de Louvain; H. Vanham, M. Van Larebeke, M. Courtibre, B. Lemaire, J. Rees, Y. Larondelle, C. Debier, Université Catholique de Louvain / Institut des Sciences de la Vie 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), o-linolenic acid (ALA, 18:3-n-3), linoleic acid (LA, 18:2-n-6) and oleic acid (18:1-n-9)) and (ii) MeHg on DHA at 5 to 10 µM on the 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 5 and 13.5 mg/kg of dry matter of diet. First results showed that fish fed with LA- and ALA-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 contrasted 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.

272 Lipids modulate cadmium toxicity in aquatic organisms A. Ferrain, UCLouvain; C. Bonhomme, Istea Lyon-Villeurbanne; I. Neefs, UCLouvain / Institut des Sciences de la Vie; V. Cornet, Université de Namur ASBL / Unité de Recherche en Biologie Environnementale et Evolutive; B. Lemaire, J. Rees, Y. Larondelle, Université Catholique de Louvain / Institut des Sciences de la Vie; K. De Schampaere, Ghent University (UGent) / Applied Ecology and Environmental Biology; C. Debier, Université Catholique de Louvain / Institut des Sciences de la Vie 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 PUFAs (polyunsaturated fatty acids, PUFAs: 18:3n-3 or 20:5n-3) or EPA (20:5n-3) significantly increased cell tolerance to an acute Cd stress while an enrichment in their n-6 counterparts, i.e. iNoloeic acid (LA, 18:2-n-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 cell surface expression and MeHg levels in AT of fish fed with FA-enriched diet contrasted 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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with 100 μM Cd. This may suggest a lower breakdown of membrane lipids in cells enriched in the three latter PUFA’s 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 A Ahad 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 daphniphyton (Daphnia magna) and the rainbow trout (Oncorhyncus 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.

273 Do specific fatty acid enriched diets protect fish against Cadmium exposure and pathogen infection?
V. Comet, Université de Namur ASBL / Unité de Recherche en Biologie Environnementale et Evolutive; A. Ouassa, R. Mandiki, Université of Namur / Research Unit in Environmental and Evolutionary Biology; A. Ferain, UC Louvain; M. Van Larebeke, B. Lemaire, Université Catholique de Louvain / Institut des Sciences de la Vie; F.E. Reyes López, Autonomous University of Barcelona / Department of Cell Biology, Physiology and Immunology; L. Tort, Universitat Autonoma Barcelona / Department of Cell Biology Physiology and Immunology; P. Kestemont, Université de Namur ASBL / Research Unit in Environmental and Evolutionary Biology

Nutrition is crucial to grow healthy fish particularly in a context of pollution, overfishing 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 Oncorhyncus mykiss juveniles to Cadmium (Cd) exposure by focusing on immune responses. Fish were fed four diets enriched with one of the following PUFA’s: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). After one month of preconditioning with PUFA’s, 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 aichromogenes (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 lysisyme and ACH50 activity for all diets except for LA 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 PUFA’s could provide 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.

274 The HERACLES Waste study: unraveling the associations between exposure to metals through diet and children cognitive functions
D.A. Sarraíjanz, Aristotle University of Thessaloniki / Chemical Engineering; A. Gotti, Aristotle University of Thessaloniki / Chemical Engineering Department / Environmental Engineering Laboratory; E. Handakas, Aristotle University of Thessaloniki / Chemical Engineering / Environmental Engineering Laboratory; S. Karakitiso, Aristotle University of Thessaloniki / Chemical Engineering

Children’s environmental exposure to metals is higher than adults, but they 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 2 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 neurophysiological and neurodevelopmental effects of such exposures. The study involved a socio- 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 cognitive performance of children. The results suggest that these factors is associated to the better quality of life, translated as nutrition of higher quality, lower exposure to environmental contamination and better educational opportunities 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)

275 The importance of ecological processes and indirect effects in determining an aquatic ecosystem’s response to ionising radiation
T.H. Hevroy, NRPA; A. Goltz, Stockholm University; L. Xie, NIVA Norwegian Institute for Water Research; C. Bradhaw, Stockholm University / Department of Ecology Environment and Plant Sciences

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. Two cosms were exposed for 22 days to a gradient of gamma radiation with 4 decreasing dose rates (20, 8, 2, 0.8 mg/ Gy). They consisted of two phytoplankton species (Raphidocelis subcapitata and Eustigmatos sp.), one zooplankton species (Daphnia magna), three plant species (Lemma 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 (Fv/Fm) 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 higher 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.

276 Impacts of radiation on DNA damage and reproduction in marine and freshwater amphipods
N. Fuller, University of Portsmouth / Biology; J.T. Smith, University of Portsmouth / School of Earth and Environmental Sciences; A. Ford, University of Portsmouth / Biological Sciences

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 (Epicaridium chevreuxi and Fukushina) respectively. Males of Echinogammarus marinus and Gammarus pulex were exposed to phosphorus-32 (P-32) at a range of dose rates from 0.0, 1.0 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.

277 Are invertebrate circulating cells inherently sensitive to oxidative DNA damage? A. Sahlmann, University of Oslo / Department of Bioscience; R. Wolf, University of Oslo; T. Hohl, Fylkesmannen i Vestfold; J. Titelman, K. Hylland, University of Oslo / Department of Biosciences

Antipathogenic 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 transient 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 and tuna were exposed to 25 and 50 μM of 10 mGy/d dose rates in

278 Assessment of DNA integrity of Palaemonidae spermatozoa using the alkaline Comet assay: a pertinent tools for environmental biomonitoring A. ERRAUD; SEBIO UNIVERSITE LE HAVRE / Sea Maritime; M. Bonnard, Université de Reims Champagne Ardenne; A. Duflot, SEBIO UNIVERSITE LE HAVRE / UMRI SEBIO; O. Geffard, Irsea / U MALY Laboratoire Ecotoxicologie; A. Geffard, Université de Reims Champagne Ardenne; J. Forget-Leray, SEBIO UNIVERSITE LE HAVRE / UMRI SEBIO; B. Xureeb, SEBIO UNIVERSITE OF LE HAVRE

The assessment 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 contaminant on sperm quality. Sperm of crustaceans displays cellular morphologies and functionalities (e.g. aflagellated and non-mobile, non-systematic occurrence of microvilli) and sperm motility that are quite different 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 (P. semisulcatus). In the last time line 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.

279 Poster spotlight: TU040, TU041, TU042, TU068

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

280 The dilemma of physical effects in aquatic toxicity tests E. Salinas, BASF SE / Experimental Ecotoxicology

A base set of freshwater aquatic toxicity tests using representative pelagic species are conducted to establish a 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 in physical effects arising from exposure to undissolved material are illustrated using examples from aquatic testing of pesticides, suspended solids, nanomaterials and dispersed polymers. While 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 routes, are solved and dissolved and by interactions 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 particle and carbon substances forming stable suspensions 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.

281 An internationally benchmarked approach towards the standardised aquatic ecotoxicity testing of nanomaterials A. Booth, SINTEF Ocean / Environmental Technology; J. Farkas, SINTEF Materials and Chemistry; K.A. Jensen, National Research Centre for the Working Environment; D. Oughton, Norwegian University of Life Sciences / Centre for Environmental Radioactivity (CERAD CoE); D. González-Gálvez, C. Delpino, LEITAT; C. Rey-Castro, University of Lleida; C. Cerrillo, G. Mendoza, IK4 Tekniker; T.F. Fernandes, Heriot-Watt University / Institute of Life and Earth Sciences; J.E. Im, University of Seoul; J. Choi, University of Seoul / School of Environmental Engineering

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 (MMNs). The key challenges identified were: (i) generation of reproducible MMN dispersions, (ii) measurement of dynamic MMN properties during standard aquatic ecotoxicity tests, and (iii) a lack of specificity in existing standard tests for MMNs. 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 MMN dispersion SOP that is performance tested using 14 different reference materials representing metal, metal oxide and carbon nanotube (CNT) MMNs. A minimum of three data sets for each MMN were generated by different laboratories using in-house calibrated probe sonicators. Measurement of the resulting MMN dispersions is by simple dynamic light scattering (DLS). The results show that reproducible MMN dispersions can be generated, both with the same laboratory and across different laboratories, for all MMNs that are suitable to DLS (i.e. granular MMNs). There are limitations with the DLS analysis in the case of high aspect ratio MMNs 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 MMN 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 MNN particle size and morphology over time, nominal and total measured MNN concentrations over time, MNN dissolution and changes in dissolution over time, and the amount of MNN lost from the exposure due to deposition. The probe sonication and MNN dispersion SOPs, together with the quantification TGD, have been applied to the testing of one silver MNN (NM-300K), three TiO2 MNNs (NM-100, NM-101 and NM-103), and three CNT MNNs (NM-400, NM-401 and NM-411) in optimised freshwater microalgae and Daphnia magna acute ecotoxicity studies.

282 Short and long-term studies to ecotoxicological impacts of engineered nanoparticles in aquatic model systems

M. Lukas, Federal Environment Agency (UBA) / Ecotoxicological Laboratory; J. Kneer, University of Bremen / Center for Environmental Research and Sustainable Technology; C. Kussatz, German Environmental Agency UBA / Ecotoxicological Laboratory

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 governmental partners that currently tries to concretize the confluence 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 (Lemma 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.

283 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 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.

284 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 polystyrene 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 mussels are mostly explained by blocking the exchange between open and interstitial water. The effects of particles and particulates on 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). Half 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

285 Use of mammalian toxicokinetic data in bioaccumulation assessment


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/PvB by REACH, as POP under the Stockholm Convention on Persistent Organic Pollutants, 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.

286 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 assessment methods to species other than fish (iii) integrating in vitro 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 of PAA 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 gastrointestinal 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 can 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 that are not covered in the illustrated firefish model. In-vivo extrapolation to in-vitro is 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 results for the group chemicals, which are (extremely) persistent, but have low aqueous solubility and slow uptake by organisms of this type. **Lesson 4:** Despite complexities associated with methodological complexities in vitro and in-vivo testing protocols, and uncertainty in the IVIVE models. **Lesson 5:** There is a need to include in-vivo methods for intestinal biotransformation to further improve the application of the IVIVE approach for bioaccumulation assessment.

**287 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 (benthos). To assess the bioaccumulation of contaminant 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 water 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 total amount 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 elasmobranchs, 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. Passive samplers 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 model approaches, in more general terms. A combination of two methods has been supported by the Czech Science Foundation Grant No. GACR 15-16512S „Investigation of accumulation of persistent bioaccumulative toxic organic substances into aquatic organisms“.

**288 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 the pelagic benthopelagic food web, detailed knowledge 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 with the observed concentrations. This highlights the benthopelagic model by factors of 2 to 7. The model performed better with the benthophagic model, for PCBs than for lipophilic contaminants. This was particularly pronounced in the benthic linkages within the model, whereas 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 bioaccumulation capacities in lower trophic levels, which are fundamental to understand also the higher trophic levels.

**289 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 4PUN) 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: *Thyamus Thymallus* and *Squalius Cephalus* whereas the lowest concentrations were obtained for the pelagic species *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 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 obtain a deterministic simulations of target compounds for twenty years in multimedia models, 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**

290 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 2009. Ecological categorization was based on the regulatory criteria for persistence (P) and bioaccumulation (B) set out in the *Persistence and Bioaccumulation*
Cyclic volatile methylsiloxanes (cVMS) are used in personal care products and are expected to be an important source for accumulation of persistent bioaccumulative toxic organic substances into aquatic environments. However, details of exposure to these substances are not well understood. The primary routes of exposure to cVMS include dermal absorption and ingestion. The USEPA has categorized cVMS as high-priority candidates for further regulatory assessment, called Categorization, for their potential to cause adverse health effects in humans and wildlife. The rationale for categorization is based on the potential for cVMS to bioaccumulate in aquatic systems and to be toxic at low concentrations.

The aquatic food web models provided as output of the IATA-based Ecological Risk Classification (ERC) approach were used to explore the role of sediments in the bioaccumulation of cVMS. The evaluation was conducted using a three-phase approach: (1) Exposure assessment, (2) Bioaccumulation assessment, and (3) Ecological Risk Characterization. The exposure assessment phase involved the use of a passive sampler to collect cVMS concentrations in water. The bioaccumulation assessment phase involved the use of a benthic linkages within the model, where several differences in biotransformation capacities in lower trophic levels, which are fundamental to the normal functioning of aquatic ecosystems, in particular to aquatic organisms. Pollution of surface waters by anthropogenic chemicals presents a significant threat to the normal functioning of aquatic ecosystems, in particular to aquatic organisms. The outcome of this study is that the IATA driven ERC would better capture chemicals that matter when the decision criteria are set to be representative of the normal functioning of aquatic ecosystems, in particular to aquatic organisms. The outcome of this study is that the IATA driven ERC would better capture chemicals that matter when the decision criteria are set to be representative of the normal functioning of aquatic ecosystems, in particular to aquatic organisms.
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’ to determine environmental ‘hazard’/‘risk’ in the environment. This methodology was developed for the purpose of assessing potential environmental impacts caused by substances considered to be of concern or on the REACH candidate list. Method 2 employs a quantitative structure–activity relationship (QSAR) approach to calculate the potential environmental impact of substances. The methods are compared for their usefulness within the context of REACH SEA. This work builds on work previously presented at SETAC. Using hexacyanoferrate(III) 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**

**295 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 Ca²⁺ concentration (in 75%), alkalinity (in ca. 30%) and pH (in more than 20%) of about of Fennoscandinavian surface freshwaters are outside the validated range of a BLM, 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 administrations about the utility 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 Biocriteria validation range. This presentation will report on an assessment of bioavailability in Fennoscandinavian freshwaters.

**296 Conceptualizing "redox equality" in ecotoxicology: the case of Cr(VI) and Cr(III)**

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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 varied. The PNECs for Cr were 5.0 to 20.25 mg/L (generally as CaCO₃, 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.

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

P. Bjerrregaard, T.G. Schmidt, University of Southern Denmark / Biology; M.P. Mose, University of Southern Denmark / Department of Biology

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 and biota at 10 stations along an 8 km stretch of the estuary 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 dw 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 dw, 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.

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

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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 contrast to 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 varied. The PNECs for Cr were 5.0 to 20.25 mg/L (generally as CaCO₃, 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.
but their variability was also large (from ca. 0.05 to ca. 0.8 kg kg\textsubscript{soil}^{-1} kg\textsubscript{metal}^{-1}). 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. Statistical analysis was assessed by weighing value scores 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.

299 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 sentinel taxa 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 ecotoxicological investigations and 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 affirming 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 straightforward 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

300 Risk to What? Translating Environmental, Social and Economic Impacts of Remediation into Stakeholder Values
S.E. Apalt, SEA Environmental Decisions Ltd; A. Fitzpatrick, A. McNally, AECOM; D. Harrison, C. Coughlan, NERA Economic Consulting; D. Edwards, B. Harrison, AECOM

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 EIS for the preferred remediation option that was proposed plan began 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 the values. Remedial options were scored by each stakeholder, using data provided by the EIS 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 pathways’ approach. Method 2 builds an impact profile/score for substances based on their environmental behavior and effects of Cr(VI). However, studies showing that virtually any review on chromium toxicity includes in its introduction a statement that chronic low level exposure to Cr(VI) has been measured as total or dissolved metal concentrations because the dissolved metal concentrations. Although only few water quality parameters are needed along 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 and future needs of the stakeholders 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 organized 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 interviews was to get a common understanding of the sustainable management of the Elbe estuary. Through a participative process, the views of >280 stakeholder groups were evaluated, documenting a diversity of priorities. The sensitivity and robustness of assessments to diverse assumptions 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 was organized with a series of three workshops with scientists, stakeholders and decision makers was developed in the project. 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303 What can Non-scientists Contribute to Marine Pollution Research?
J. Brant, Cefas; L. Gilliam, eXXpedition; J. Jambeck, University of Georgia; A. Kaereman, MTM Research Centre, Oebro University; O. Lane, Biodiversity Research Institute; A. Barrows, Adventure Scientists; D. Papouliaas, E. Penn, eXXpedition
Although ‘citizens’ have undertaken data collection for many years as volunteers, there is still apprehension from many scientists about the value of 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 numbered on board and responsible for plastic waste 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.

304 The use of photography in science communication - Its potential and its challenges
A. Bonisoli Alquist, 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 espoused 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

305 Dealing with variability in ecosystem vulnerability for environmental management
M. Zip, RIVM / DMG; L. Posthuma, RIVM / Centre for Sustainability, Environment and Health; S. 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 distribution (EVD): 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 cytotoxicity. Deriving and using EVDs in an operational way is important 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.

306 One Planet Thinking: Towards companies that perform within the earth’s regenerative capacity
A. Krutch, 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 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.

307 Absolute environmental sustainability assessment using new Planetary Boundaries based LCA methodology: framework and a case study
M.W. Ryberg, Technical University of Denmark / Quantitative Sustainability Assessment Department of Management Engineering; M. Owsianik, Technical University of Denmark; K. Richardson, University of Copenhagen; M. Haußchild, 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 incorporating ES into risk assessment and management requires operationally, incorporation of ES into environmental risk assessment and management has been growing. Ecosystem Services (ES) are the services that ecosystems provide to human societies. Ecosystem Services in Retrospective Risk Assessment, Remediation and Sustainability Management is 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-LCA 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.

308 Absolute environmental sustainability assessment using new Planetary Boundaries based LCA methodology: framework and a case study
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309 Absolute sustainability assessment of product life cycles through calculation of carrying capacity entitlement at industry level
G. Majeau-Bettez, CIRAIG - Polytechnique Montréal; A. Bjorn, Polytechnique Montréal / CIRAIG; R. Wood, Norwegian University of Science and Technology / Biology; M. Margni, CIRAIG - École Polytechnique de Montréal / Mathematical and Industrial engineering; C. Bulle, CIRAIG - ESG - UQAM / Strategy & corporate social responsibility

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 soy milk and cow 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 freshwater 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 sustainable part is measured over a 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. One approach 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.

310 Ecosystem services from a regulatory/risk management prospective
K.M. Nienstedt, European Commission - DG SANCO / PPR

311 Chemicals: Assessment of Risks to Ecosystem Services (CARES). Where are we now and where are we going?
L. Malby, The University of Sheffield / Dpt. of Animal & Plant Sciences; S. Marshall, Unilever / SEAC; J. Faber, Alterra; P. van den Brink, Alterra and Wageningen University

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.

312 Ecological production functions: key attributes and challenges of implementation
R. Bruins, U.S. EPA / Ecological Exposure Research Division; T. Canfield, U.S. EPA / ORD / NRMRL; GWERD; C.S. Duke, Ecological Society of America; L. Laplace, LHJ Consultancy; A. Naidu, Landscape Protection Agency; F. Schurer, University Koblenz Landau / Institute for Environmental Sciences

Ecological production functions: recent developments; 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.
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 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.

314 Panel discussion

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

315 Zebrasfish transcriptions after chemical exposure - a quest for common grounds
A. Schuettert, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology; K. Reiche, Fraunhofer Institute for Cell Therapy and Immunology / Bioinformatics Research Unit Department of Diagnostics; R. Altenburger, UFC Centre for Environmental Research / Bioanalytical Ecotoxicology; W. Busch, Helmholtz centre for environmental research - UFZ / Bioanalytical Ecotoxicology 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 BTR expert workshop. Arch. [2] Williams TD et al. 2014. The toxicological application of transcriptomics and epigenomics in zebrafish and other teleosts. Brief. Int. Genomics 13:157–71 [3] Villeneuve DL et al. 2014. Adverse outcome pathway (AOP) development I. Strategies and principles. Toxicol. Sci. 142:312–320

316 Transcriptomic approach in microalgae and macrophyte in-situ exposed in river impacted by chlor-alkali plant effluents
C. Cosio, Geneva University / Department F.A. Forel of environmental and aquatic sciences; P. Dranguelt, University of Geneva / Department FA Forel for Environmental and Aquatic Sciences; S. Le Faulcheur, Institute F.-A. Forel, University of Geneva / Department FA Forel for Environmental and Aquatic Sciences; R. Beauvais-Fleuret, A. Altmann, I. Worms, University of Geneva; V. Slaveykovska, University of Geneva / Department F.A. Forel of environmental and aquatic sciences
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 microalgae Chlamydomonas reinhardtii and the macrophyte Elodea nuttallii were exposed 2hn 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 microalgae 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. Transcriptome 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.

317 Elucidating the toxicity of the flame retardant tris (2-butoxyethyl) phosphate (TBP) by transcriptomics
B. Krivoshiev, Systemic Physiological and Ecotoxicological Research group University of Antwerp; G. Beemster, K. Sprangers, B. Cuypers, K. Laukens, R. Blust, University of Antwerp; S. Husson, University of Antwerp / Biology Tris (2-butoxyethyl) phosphate (TBP) 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. TBP 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 TBP 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 TBP inducing proliferation at lower concentrations. Treatment with cub-cytotoxic concentrations of TBP 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 TBP 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, TBP was shown to alter steroid hormone biosynthesis, thereby highlighting its potential endocrine disruptive effects. Transcriptomic data provide the foundation for the first such study investigating toxicogenomics of TBP in human models, and agrees with the adverse health effects seen in other organisms.

318 Daphnia magna transcriptomic profiling of chemically induced starvation responses highlights new molecular fingerprints with regulatory potential.
B. Campos, IDAEA-CSIC / Environmental Chemistry; C. Barata, CSIC / Environmental Chemistry 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 feeding impacts of chemicals. Understanding the effects that are the molecular specific fingerprints of exposure. In this work we exposed Daphnia
magneto six contaminants of different chemical properties (Cadmium, Copper, Cyalothrin, Fluoranthrene, Lindane and microcystins from live Plankothrix 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 oxidative damage in zebrafish brain. The findings of this study suggest that a diet deficient 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

Environmental Levels of Anxiolytic Pharmaceuticals Alter Migration of Atlantic Salmon in Both Lab and Field

T. Brodin, G. Hellstrom, Umea University / Department of Ecology and Environmental Science; J. Fick, Umea University

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 known, 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 can disturb 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 consequences. In the current research we performed a comprehensive, large-scale studies in lakes and rivers using acoustic telemetry to test if findings from the lab also hold in natural settings. Here we 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 pharmacologically 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 reach. In the field. The observed studies suggests that effects of pharmaceutical contamination of natural systems might be much more widespread than we predict based on conventional ecotoxicological tests.

322 Developing high-throughput, multi-endpoint, behavioural assays for model crustaceans

S.A. Kohler, University of Portsmouth / Biological Science; A. Ford, University of Portsmouth / Biological Sciences; M.O. Parker, University of Portsmouth

Behavioural and ecological responses of invertebrate species to exposure to environmental contaminants are increasingly being used in ecotoxicological testing. Recent advances have facilitated the use of high-throughput approaches to test these endpoints. Developing novel, high-throughput assays for crustacean species is required to test the impacts of pharmaceuticals on these taxa. In this study, a new assay was developed using the brine shrimp Artemia sp. to test the effects of pharmaceuticals on a range of endpoints, including growth, survival, and physiological responses. The assay was validated using a range of pharmaceutical compounds, and the results were compared to traditional methods. The new assay showed promise as a high-throughput tool for ecotoxicological testing, and further development of this method may lead to improved understanding of the effects of pharmaceuticals on crustacean species.

321 Environmental Levels of Anxiolytic Pharmaceuticals Alter Migration of Atlantic Salmon in Both Lab and Field

T. Brodin, G. Hellstrom, Umea University / Department of Ecology and Environmental Science; J. Fick, Umea University

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 and is excreted as fluoxetine by the human body. As such, fluoxetine is ubiquitously present in both treated and untreated wastewater effluents. The objective of this study was to investigate the effects of fluoxetine exposure on the behaviour of Atlantic salmon (Salmo salar) in the lab and field. The study was conducted in both indoor and outdoor experiments, with the goal of determining whether fluoxetine alters the migration behaviour of salmon. The results of this study suggest that fluoxetine exposure can significantly affect the migration behaviour of Atlantic salmon, with implications for population dynamics and conservation efforts.
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 effect of fluoxetine on avian courtship, we conducted an in vivo chronic effects study in which we exposed wild caught Eurasian starlings (Sturnus vulgaris) to an environmentally relevant concentration of fluoxetine. To measure the effects of exposure, we focused on a combination of behavioral 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 as well as physiology, it could have implications for breeding success in wild birds and may be a factor in observed population declines.

324 Arbitary experimental methodologies are influencing study outcomes in behavioural toxicity testing
S.D. Melvin, M. Deviagna, 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, tests that offer 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 toxicity. 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)

325 Availability and organization of QSAR-s influences the higher understandability and acceptability of descriptive and predictive models
U. Maran, Institute of Chemistry, University of Tartu / Department of Chemistry; S. Sild, G. Pir, A. Garcia-Sosa, Institute of Chemistry University of Tartu; I. Kahn, Tallinn University of Technology

Standard QSAR databases are insufficient. The QRDB database includes QsarDB and Fujita, QsarDB 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 reproducible. This hinders the understandability and acceptability of descriptive and predictive models. Fortunately 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 dataformat [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 each model, endpoints and QsarDB data format for chemical substances 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 36:40. 2. Sild S, Pir G, Garcia Sosa AT, Maran U. 2015. Chem Res Toxicol 28:768-774. 3. Setac Europe 27th Annual Meeting Abstract Book. 4. IUCN 2014. J Cheminfor 6:25

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326 Data integration to increase quality and reliability of Qsar predictions
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R&D in EC requires information on 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 predictions often require the use of different and independent intelligent models 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 (Demeter-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 classifications 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.

327 CATALOGIC 301C model - validation and improvement
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The growing integration of chemistry in the modern daily life put foremost 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 regional inventories and substances of substances of the technical or non-test methods was recommended. In Europe, REACH legislation encourages the use of alternative in silico methods such as Qsar models. According to the recent progress of CSCL in Japan, Qsar 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 are 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 CSCL 301C model. Significant improvements compared to the previous values of test chemicals in 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 persistency prediction but also on prediction of degradation products. Validation results showed that for both, prediction of ready and 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 metabolism 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 for documented degradation pathways. The current training set of the model contains approximately 2580 observed BOD values and documented degradation products for about 750 chemicals.

328 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 Bioaccumulation Factor (BAF) on fish. In order to develop a QAR model 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 gain 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 BAF, 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 BAF predictions and the descriptor interpretation allowed investigating and rationalizing the structural features responsible for the bioaccumulation of organic chemicals within the food web.


329 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 ecorecological 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 (HAGS) 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 probability of phase II metabolic prediction “in silico Contras 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

330 Analysis and ecotoxicological investigations of poorly soluble chemical 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. Within the REACH framework, ecotoxicology tests are mostly 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 (ECOSOM) was to develop ecotoxicological methods and suitable analytics for testing highly hydrophobic substances, followed by a critical evaluation of the “poorly soluble approaches” 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 (ETN<sub>acid</sub>) do not exhibit any acute or chronic aquatic toxicity (cf., hydrophobic shock). Dose-benzene (DBB) 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 DBB 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 (6×10 to 2×15 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 soluble approach” and reported hydrophobicity cut-off in toxicity.

331 Should regulatory short-term aquatic toxicity tests be waived for highly hydrophobic chemicals with low 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 following standard acute ecotoxicity tests at three trophic levels: algae, invertebrate, and fish. Model suggests that chemicals with an octanol–water partition coefficient (K<sub>ow</sub>) greater than 10<sup>6</sup> 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<sub>ow</sub>. Considering uncertainties of the model, we propose a revision of data requirements for highly hydrophobic chemicals with log $K_{ow}$ > 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.
The impact of mixtures of dissolved phases such as surfactants and humic acids (HA) on highly hydrophobic contaminants (hHOCs, log Kow > 5.5), many of which can be highly toxic e.g. dibenzop-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 offsite transport of contaminants. Therefore, methodologies to quantify the magnitude of such effects need to be developed. The current work focuses on the biodegradation behaviour of a binary anionic (C12-2-LAS)-nonionic (C12EO4) surfactant mixture at two mass ratios (33.67 or 83.17 C12EO4:C12-2-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. Technical surfactants are predominantly produced as mixtures of surfactants and are considered to contribute to the purity of surfactant mixtures with HA increased with increasing HA concentration. PCDD and PCB behaviour of a binary anionic (C12-2-LAS)-nonionic (C12EO4) surfactant mixtures in fields such as toxicity testing or bioavailability determination of hHOCs in the environment. 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. Adam IKU, Rein A, Miltner A, da Costa FAC, Trapp S, Kästner M. 2014. Experimental results and integrated modelling of bacterial growth on insoluble hydrophobic substrate (phenanthrene). Environ. Sci. Technol. 48 (15), 8717-8726. Rein A, Adam LKU, Miltner A, Brumme K, Kästner M, Trapp S. 2015. Impact of bacterial activity on turnover of insoluble hydrophobic substrates (phenanthrene and pyrene) — model simulations for prediction of bioremediation success. Journal of Hazardous Materials, 306:105–114. Advances in Soil Ecotoxicological Risk Assessment of Chemical Stressors (I)

333 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 measuring 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 and dissolved organic carbon (KOC and 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 (KOC) and dissolved organic carbon/water (KDOC) 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, D6, D8) and linear (n) VMS are consistent with some earlier 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 (DHfC) determined for VMS from measurements of KOC at temperatures between 5 and 30°C, but also from a high affinity for organic carbon at lower temperatures. Measured of the enthalpies of phase change between water and octanol (DHfC80) show the opposite trend, ie, lower affinity for octanol at lower temperatures. The temperature dependence of KOC is thus qualitatively different than that of KOC for the VMS that it the enthalpies of opposite signs. Potential future applications to other hydrophobic substances, including mixtures that are classified as UV-CBs (unknown or variable composition, complex reaction products or biological materials) under the European Chemicals regulation REACH will be discussed.

334 Simulation of bioremediation options by microbial degradation of aged (PAH) contaminations in soils

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Many at 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 works sites. In order to find a usable biological 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. Adam IKU, Rein A, Miltner A, da Costa FAC, Trapp S, Kästner M. 2014. Experimental results and integrated modelling of bacterial growth on insoluble hydrophobic substrate (phenanthrene). Environ. Sci. Technol. 48 (15), 8717-8726. Rein A, Adam LKU, Miltner A, Brumme K, Kästner M, Trapp S. 2015. Impact of bacterial activity on turnover of insoluble hydrophobic substrates (phenanthrene and pyrene) — model simulations for prediction of bioremediation success. Journal of Hazardous Materials, 306:105–114.
Ecology; S. Gutierrez, ECHA-European Chemicals Agency / Biocides; C. Carlon, P. Karamertzis, European Chemicals Agency - ECHA; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division; M. Claessens, Chemnour; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; A.D. Redman, Exxon Mobil Biomedical Sciences / Exoenvironment and Climate Science Division; A. Kapunan, European Chemicals Agency - ECHA

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 tier 2 method in the new REACH Directive, it may be used for 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 PNECsoil 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 validation purposes was assessed. This underscores 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 BPR to support the screening of the new REACH cardiovascular diseases, University of Life Sciences; I. Horetzky, University of Life Sciences / Department of Life Sciences of University of Voimbra; I. Sundh, Swedish University of Agricultural Sciences; A. Tiktak, PBL Netherlands Environmental Assessment Agency / Water Agriculture and Food; C. Topping, The Panel on Plant Protection Products and their Residues (PPR) has been tasked by the European Commission to revise the Scientific Evaluation of the soil ecosystem (soil protection goal SPG) 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, EFSAs 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 scientific opinion. The working document, which has jointly 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. In order to overcome 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.

337 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 Conceptual framework to select PGs in consultation with risk managers, considering a cost-benefit evaluation of the associated socio-economic 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 be considered, which is expected to demonstrate 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 range’ for in-field crop protection (the Cultural landscape PG) from 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 difficult to evaluate if the in-field PGs should be based on soil parameters or using semi-field trial test. Currently, the models PRAESS and ChinaPEARN, which developed by NIES and ICAMA in China, are applicable to pesticide-exposure concentration (PEC) in soil specific by considering specific scenarios in China. Proposed test systems for effect assessment include acute toxic concentration or reproduction of earthworm, reproduction test, nitrogen transformation test, litter bacterial and earthworm field test. The PNEC can be calculated using the end point obtained from ecotoxicological studies and corresponding uncertainty factors (UF). Tier 1 risk assessment mainly focuses on the effects of acute or chronic toxicity. The next step of the PRAESS assessment and N - transformation assessment. High tier risk assessment mainly
focusses 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)

340 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, hereafter ‘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 process, 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 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.

341 The use of reference materials in microplastic research: general aspects
K. Altmann, BAM- Federal Institute Materials Research and Testing; A. Barthel, C. Bannick, N. Obermaier, UBA Umweltbundesamt; F. König, M. Jekel, TU Berlin; R. Becker, U. Braun, BAM- Federal Institute Materials Research and Testing 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 exist. 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), polylamide (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 prolated sphericals. These can be available at cost-efficient 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 appropriate, 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 production of the microplastic particles is the production of polymer pellets in extrusion (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.

342 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 of different particle sizes, 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 sampling time and number of extractions need to be standardised to allow 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.

343 Analysis of microplastics by LC-QExactive Orbitrap in coastal areas of Catalonia
G. Schirinzì, Institute of Environmental Assessment and Water Research IDAEAC/CSIC; M. Llorca, IDAEA-CSIC / IDAEA; M. Farre, IDAEA-CSIC / Environmental Chemistry; D. Barcelo, IQAB-CSIC / Dept Environmental Chemistry

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 nanoparticles (NPLs) and microplastics (MPLs)). According 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]. MPLs are a part of 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).

References

344 Separation and identification of tyre debris in environmental samples
P.E. Kloeckner, Helmholtz Centre for Environmental Research GmbH - UFZ / Analytical Chemistry; S. Wagner, Helmholtz Centre for Environmental Research GmbH - UFZ / Department of Environmental Geoscience; T. Reentmsa, Helmholtz Centre for Environmental Research / Department of Analytical Chemistry

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 analyzed via elemental analysis (pre-concentration via mechanical stirring) 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 date a recovery of 94.7% for the pre-concentration and density separation with sodium pyromontstate. 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 insights into the elemental composition. The reduced sample 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**

345 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 first step parameter for a grouping of ENM in air, water and sediment/soil regarding their transformation or mobility were identified 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 matrix. This allows a first assessment of the adsorption and aggregation behaviour will be studied as grouping parameter 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 Ministry for Education and Research (BMBF) under grant no.: 03XP002

346 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. 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. In the case of H and NO3 concentration in the media, the agglomeration / adsorption and sedimentation behaviour will be studied as grouping parameter 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 Ministry for Education and Research (BMBF) under grant no.: 03XP002

347 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 socio-economic analysis to help its users understand 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-Sq 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.

348 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 governments also demand for risk assessment and management. Nanotechnology is often known for its 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 and emerging nanotechnologies. This is the basis for the development of risk assessment tools and models to support decision making. The RATA project is a European Networking Project. The project is about putting RRI in practice within a large Dutch nanotechnology consortium, as an example of putting RRI in practice as integrated part of a research program.

349 Poster spotlight: WE185, WE186, WE187, WE190

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

SETAC Europe 27th Annual Meeting Abstract Book
350 Development of a new industry lead Life Cycle Inventory for cobalt and perspectives on the metal LCA harmonisation effort
J.M. Frv, ERM; C. Pettit, Cobalt Development Institute / Regulatory & Sustainability; S. Amonier, 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 recording methods. The CDI has obtained, and is assessing, the interconnectivity of the 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 first 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.

351 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 just released 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 impact 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.

352 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 with an increasing reliance on critical or geologically 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 impacts. Critical and scarce metals are 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) fully 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 geographically 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.

353 Comparison and improvement of life-cycle-modelling methods for recycling
Z. Jiang, H. Wang, Sichuan University / College of Architecture and Environment; S. Wang, 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 inapplicable, allocation principles. 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 methodology 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 developed 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.

354 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 environmental impacts of recycled YOX compared to primary YOX. Several goal definitions had been formulated, which all aimed 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 assessment due to June 2017. Among the 11 descriptors which describe what the environment will look like when the goal is achieved, the one corresponding to contaminants is the descriptor 8 "risk assessment and management of waterbodies (ground, surface, drinking and marine waters)."

355 Risk assessment and management of waterbodies (ground, fresh, marine and drinking waters)
SETAC Europe 27th Annual Meeting Abstract Book
Active biomonitoring using the freshwater crustacean Gammarus fossarum: an operational tool to monitor chemical contamination and toxicity in continental surface waters

G. Jubeaux, R. Recouvra-Massuquant, BIOMAE; F. Massat, La Drôme Laboratoire; J. aunot, O. Geoffard, Irstea / UR MALY Laboratoire Ecotoxicologie

The use of biomonitoring tools, such as crustaceans, is 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 exposure 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) introduces aquatic ecotoxicological effect quotients (EQs) 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).

356 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, larva 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, perethrin, ethan, 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 larval behaviour. 3D exposure of aquatic effects on Environmental Quality Standards (EQSs) and perethrin. 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 should be part of a more integrative approach of chemicals regulating aquatic ecosystems. 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.

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

R. Sijsers, KWR Watercycle Research Institute / Chemical Water Quality and Health; P. Stuyfzand, KWR Watercycle Research Institute / Geochemistry; L. Pijker, A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health

Pesticides could enter surface and groundwater systems 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 were quantified based on a detection limit of 6 ng/L (3,634 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). Depending on the local land-use, soil and water system characteristics these compounds are relevant for aquatic ecosystems. Monitoring programs of pesticides in drinking water, sustainable water management and mobile recent active substances, to signal and manage the risk for the ecological environment or for human health. 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 should be part of a more integrative approach of chemicals regulating aquatic ecosystems. 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.
Barcelona). PAH, PCB, heavy metals (e.g. Cd, Pb, Hg) concentrations in mussels and sediments were obtained from a 30-year French observatory program (ROCC) 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 mussels 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)

360 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 sustainable 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. Poison 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.

361 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. Beine of Gennia, European Commission - DG Environment
Aim in 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), Ökopel 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. In Methods in 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. In Results 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. In Conclusions it is indicated 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.

362 Identification, prioritization and evaluation of potential New Emerging Risk of Chemicals (NERCs) for the environment
J. Bakker, National Institute for Public Health and the Environment (RIVM) / VSP; Y. Brünen de Bruin, European Commission - DG Joint Research Centre; E. Hogendoorn, RIVM / VSP
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 notification of these substances through environment policies and the environmental 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.

363 Establishment of the European Exposure Strategy 2025: Common interests of SETAC Europe and ISES Europe for picking up, sharing and communicating signals
Y. Brünen de Bruin, European Commission - DG Joint Research Centre; J. BESSSENS, European Commission, DG JRC Joint Research Centre
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, NGOs, employers, CEOs, competent authorities and stakeholders, 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.

364 Panel discussion
21st century Ecotoxicology and Human toxicology: Applications and perspectives for the use of OMIs data (II)
Environmental endocrine compound concentrations and human and ecosystem health effects

370 Assessment of chronic effects of chemicals and detection of potential endocrine-disruptors for a hermaphroditic mollusk: possible endpoints in a full life-cycle bioassay
C. Dierick, University of Bourgogne Franche-Comté; F. Gimbert, University of Franche-Comte / UMR ChronoEnvironnement; R. Scheifeier, University of Franche-Comte / Chrono-Environnement; A. de Vlaeury, University of Franche-Comte / Department of Chrono-Environnement 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-cycle tests measuring apical endpoints such as fecundity that can respond to both EDCs and some non-EDCs have been recently published for freshwater mussels. 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 repro toxicity in mussels are not known. For the terrestrial environment, few life cycle 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 hermaphroditic 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 asperus, allowing exposure from embryogenesis to reproduction, was used to assess the effects of Bypa@, 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. Bypa@ had no consistent endocrine disruption effects, with a significant enhancement of growth speed followed by a marked reduction of the fertility of C. asperus snails. The horneric effect of GlyBH on growth could result in 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 hermaphroditic organisms.

371 Steroid and thyroid gene transcription ontogeny during the first 32 days of zebrafish development improves our understanding of endothocrine disruption J. Periz Stanacev, E.D.Michiels, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; E. Stückens, University of Antwerp; I. Gabriëls, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; C. Pype, University of Antwerp / Applied Veterinary Morphology, Dept of Veterinary Sciences; E. Verbeuken, University of Antwerp / Applied Veterinary Morphology, Dep of Veterinary Sciences; S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology, Dept Veteranary Sciences; A.L. Van Nuijs, University of Antwerp / Toxicological Centre, Dep of Pharmaceutical Sciences; L. Vergauwen, D. Aepen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences Effects of endothocrine 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 morphological effects of EDCs, we can hypothesize and validate molecular mechanisms of endothocrine disruption. Studies using zebrafish embryos reported a wide range of effects on 17α-ethinylestradiol (EE2), including skeletal malformations, pericardial and yolk-sac oedema and an uninflected 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 steroidogenesis pathway and 9 genes related to the thyroid metabolism, were measured by qPCR. Second, we created an overlay of the ontology 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 analyses show that the expression of selected genes is strongly reduced the number of clutches, possibly due to a permanent eversion of the penis. Thymus development was not affected. This dataset can help detecting endocrine-disrupting chemicals (EDCs) is recognized. Partial life-cycle tests measuring apical endpoints such as fecundity that can respond to both EDCs and some non-EDCs have been recently published for freshwater mussels. 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 repro toxicity in mussels are not known. For the terrestrial environment, few life cycle 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 hermaphroditic 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 asperus, allowing exposure from embryogenesis to reproduction, was used to assess the effects of Bypa@, 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. Bypa@ had no consistent endocrine disruption effects, with a significant enhancement of growth speed followed by a marked reduction of the fertility of C. asperus snails. The horneric effect of GlyBH on growth could result in 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 hermaphroditic organisms.

372 Combined exposure to EDCs resulting in neurodevelopmental disorders D.A. Sarigianis, Aristotle University of Thessaloniki / Chemical Engineering; K. Polanska, W. Hanke, Nofer Institute of Occupational Medicine; A. Sulitgolou, A. Gabriel, Aristotle University of Thessaloniki / Chemical Engineering; E. Handakas, Aristotle University of Thessaloniki / Chemical Engineering; E. Handakas, Aristotle University of Thessaloniki / Chemical Engineering / Environmental Engineering Laboratory; S. Karakitsios, Aristotle University of Thessaloniki / Chemical Engineering It has been well established that organic compounds such as phenols, PCBs and pesticides 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, BBP 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 associations were found between the exposure to the metabolites of DEHP, BBP and DiNP and the cognitive development of the children. The analysis of the association with MetS, but to a lower degree (90% of probability). This is the first evidence that the effects of environmental contaminants, such as POPs and metals, persist through the developmental stages and that the prenatal exposure has the greatest impact on cognitive and motor development. This is an important finding for the development of future intervention strategies. The authors conclude that the study results are important for the understanding of the complex contribution of environmental contaminants to the development of neurological disorders in children.
environment. Hence, the MiSSE project (Mixture aSSession of Endocrine 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 sentinel for hormone disruptor indoor exposure and their thyroidogeic 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 perfluoralkyl substances (including the phosphates), whereas dust was also analyzed 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 TCPP are dominating the chemical profile of the dust whereas the bacteria levels 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 ambiguous 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).

374 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 chemicals usually produced for agricultural and industrial applications, and possess notable toxicity potential and persistence. We pose a threat to human and the environment. Exposure to POPs has been related to increased risk of some diseases such as diabetes and most recently, Metabolic Syndrome (MetS). Metabolic Syndrome is a group of risk factors characterized by central obesity, dyslipidemia, glucose intolerance and arterial hypertension. 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 triglycerides 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), polychlorinated (PCBs) and polybrominated (PBDEs) ethers. 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: Unraveling behavioural responses to aid environmental and regulatory toxicity (II)

375 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 fish species, 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, and this behavior is affected by multiple oil spills in fish populations. 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 larval life stages. We developmentally exposed sheephead minnow (Cyprinodon variegatus variegatus) to water accommodate 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 8-9 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 sheephead 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.

376 High throughput sequencing and behavioural endpoints highlight effects of antipodeants on crustaceans at environmentally relevant concentrations

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The effects of antipodeants 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 antipodeants can effect behaviour in scototaxis/photoraxis and activity based experiments. Here we present the results of several repeated behavioural trials exposing gammarid amphipods (Echinogammarus marinus & Gammarus pulex) to antipodeants linked to gene expression using next generation sequencing. The animals were exposed to these five antipodeants at environmentally relevant concentrations from 1 to 1000 ng/L during short-term (1 hour and 1 day) and medium-term (developmentally exposed sheephead days) experiments. The performance of the amphipods was tracked using the behavioural analysis software during 12 min alternating dark/light conditions. Antipodeant 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 citalopram (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 neurodegenerative 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.

377 Persistent Organic Pollutants chronic dietary exposure disrupt behavioral and molecular responses over several generations in unexposed zebrafish (Danio rerio)

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Persistent Biphenyl Dibenzoates and Polychlorinated 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, zebrafish 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 (2C8= 1974.48 ng g⁻¹ and 2SBD= 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 in F3 and F1 generation. In F1 the challenge of the novel tank assay was correlated with the monoamines analyses which 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.
Pharmaceutical alters mate choice and gene expression of a fish

M. Sariano, B.B. Wong, m.g. bertram, c.p. johnstone, Monash University / School of Biological Sciences; M. Allinson, The University of Melbourne / School of Chemistry; j.a. craft, Glasgow Caledonian University / Health and Life Sciences Aquatic habitats are increasingly being exposed to chemicals that, even at low levels, can affect the growth and morphology of aquatic organisms. The objective of this study was to investigate the impacts of short-term exposure to selected pharmaceuticals on the reproductive behaviour of the guppy, Poecilia reticulata. The study was conducted in a controlled laboratory setting, with fish exposed to increasing concentrations of the pharmaceuticals. The results showed that exposure to the pharmaceuticals significantly altered the mating behaviour of the guppies, with a decrease in the number of spawning events and a change in the female choice of males. These findings highlight the potential impacts of pharmaceuticals on aquatic ecosystems and the need for further research to understand the long-term effects of exposure.

Use of TK/MD 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 use of endpoints. Additionally, the CLP-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 Pesticide Environmental concentrations (Predicted Environmental Concentrations) values. Higher tier risk assessment 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 scenario. Furthermore, in aquatic chronic experiments, it is performed that can not be conducted indefinitely. TK/MD models, based on experimental data can be used to model biological responses to these different exposure conditions. TK/MD models could become a very useful 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, developed within academics, industry and regulators could be developed and widely used in a harmonised way for active substance monographs and/or for registration of plant protection products.

379 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 conduct field tests to evaluate the ecological relevance of the avoidance toxicity assays (e.g. LC50, or EC50). Avoidance behavior to a gradient of salinity was studied for the following species: Daphnia magna, Danio rerio, Heterocyclops 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 LC50 values and EC50 values were plotted in Species Sensitivity Distribution curves (SSD) and the Hazard Concentrations (HC5) were computed and compared. Results showed when species are confronted with a saline gradient, 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 AC50 value of 4.99 mScm-1. The LOEC 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 LC50 and the AC50 (4.37 and 11.68 mScm-1, respectively). Computed HC5 from SSD curves were of 7.34 (4.49-12.0), 3.43 (1.84-6.07) and 3.41 (1.87-6.07) for D. magna, X. laevis and D. rerio, 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 (I)

380 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 (TK/TKD) model approach. In this modelling study, we use the General Unified Threshold model for Survival (GUTS), working with a case study on propiconazole in Gammarus pulex, and provide recommendations for the evaluation of TK/MD models.

381 Linking the Adverse Outcome Pathway to Dynamic Energy Budgets: A 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 ‘quantitative’ aspect 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 (TK/MD) models. In contrast to the higher-level models, TK/MD 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 TK/MD models is more like a complex interconnected web. These aspects have consequences for the application of frameworks for model evaluation to TK/MD 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 TK/MD models.
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.

383 Linking the Adverse Outcome Pathway to Dynamic Energy Budgets: A conceptual model and two case studies
R.M. Nisbet, UCSB; C.A. Murphy, Michigan State University / Fisheries and Wildlife
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 describe these processes, 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 was 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.

384 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 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 variety of complex disrupting stressors, 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)

385 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 not be a concern for other persistent halogenated flame retardants. However, recent research has shown that OPEs can persist 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 nine alkyl/aryloxyaryl PEs. PEs with thicknesses of 50µm, 800µm, and 1600µm were deployed from July 2012 to July 2013 and from June 2014 to July 2015 at depths ranging from 248 to 2353 m. PEs 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 chlorineated OPEs (tris(1-chloro-2-propyl)phosphate (TCP) and tris(1,3-dichloro-2-propyl)phosphate (TDCIPP)) made up >70% of total OPEs in all samples. Dissolved concentrations of TDCIPP were estimated to range from below detection limits (<1DL) to 465 µg/L, while for TDCIPP concentrations ranged from 8 to 38 µg/L. Among the non-chlorinated OPEs, tri-n-butyl phosphate (TBP), tri(n-propyl)triphenyl phosphate (TPTH), 2-ethylhexyl diphenyl phosphate (EEDPP), and tris(2-ethylhexyl)phosphate (TEHP) were frequently detected, with TBP at the highest concentrations, ranging from

386 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 (POCS) and Chemcatcher have received increasing attention in environmental research as they allow the measurement of more ecotoxically 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-sorpive 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.

387 Passive sampling as a screening tool for determining the influence of hospitals on emissions of pharmaceuticals
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The occurrence of pharmaceuticals in the environment is an emerging concern, and with both the ageing and the increasing use of prescription drugs, increasing pressure on the aquatic environment and consequently emissions to the aquatic environment will most probably increase. In the forthcoming decades there will also be a shift in use from developed to so-called pharmerging 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 municipalities in the western Netherlands was determined. Next to this, the removal rate of teh different pharmaceuticals on teh STP were determined for this. We deployed passive samplers (speedisks) on different locations int the sewage sytem for a number of days. After deployment, the samplers were extracted and analysed on total 80 pharmaceuticals, divided in antibacterials, antivirals, antidepressants, antiinflammatories, anticoagulants, anti-diabetics, antihypertensives, and finally antiemetics. Based on earlier results, we were able to calculate
aceous 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, probably caused by the different application patterns within the hospitals. Based on the load calculations, the hospitals appeared to be the main source, but for most compounds, other sources like households and nursing homes appear 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. These findings 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.

388 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). Combined with posing with high-end ultra-high-performance liquid chromatography 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 see whether to non to polar organic micropollutants in the marine micropollutants (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-HRMS 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 micropol lutants, 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 micropol lutants. Non-target screening demonstrated additionally that 14 emerging organic pollutants co-occur at each sampling location. 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.

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

M. Reininghaus, RWTH Aachen / Biogie 5; N. Niehus, H. Hercht, University of North Sea - Coastal Marine Sediments - Comparing in situ versus ex situ experiments we examined (i) spatially resolved freely dissolved PAH concentrations; (ii) baseline toxicity potential on the basis of chemical activities (iii) site specific mixture compositions and (iv) site specific distribution ratios. Advances in Soil Ecotoxicological Risk Assessment of Chemical Stressors (II)

390 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 contaminants 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 included 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 HCIB (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.

391 Toxicity 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 streamline 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 organometallic (copper dimethyldithiocarbamate); a water-soluble inorganic salt (CuSO4); a water-insoluble inorganic (CuO); a water-insoluble organic metal salt (copper D-glucenate); and a water-insoluble organic metal salt (copper glucelolate). 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 Cu2+ 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 depth to the risk assessment process.
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 organometallics.

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

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Soil microorganisms, invertebrates and plants play an essential role in ecosystem services. The inhibition of microbial functions function into environmental regulations is at early stages. 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 life. 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. Soil samples containing non-sterile soils were dosed with 2,500 mg/kg of a mixture containing 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) Nutrient cycling, (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 emissions, 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 oribited mite inhibition in these same soils to link microbial and ecosystem service endpoints to a soil toxicity test as well.

393 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 compared to other methods. One useful feature of avoidance tests 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, Opisthobius 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 10,000 mg/kg which is well above the threshold for reproductive effects in collolumban species.

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

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Biofungam is considered a good alternative to chemical fungamtion 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 biofungamns. Efficacy of both chemical and biofungamns on target species have been well documented, however, very little information is available on its effects on non-target species. Positive 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 biofungamns, broco, mustard and oilseed radish, and two chemical fungamns, metham sodium and cadusafos, were investigated for possible effects on non-target and essential soil organisms. The ecotoxicity of fungamns 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 colonincs. Changes in the soil microbial community function and structure were evaluated by means of Biomis™ Ecopeats and phosphohlipid 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 fungamns 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)

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


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 freshwater, 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 plastics in freshwaters. Only roughly estimates of MP and mesoplastics in freshwaters is just at the beginning. Sor far, potential effects of additives and adsorbed pollutants 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.
Integrated approaches for linking chemical contamination with biological effects

400 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 mainly 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 biochemical analysis 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 active sampling of water from the Danube river differed depending on the studied endpoint 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).

401 Effect-directed analysis (EDA) of Danube River water samples receiving untreated municipal wastewater from Novi Sad, Serbia.

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Abstact: 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 (LV-SPE) fractionation. The active fractions were subjected to multivariate 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), progesterone (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 fractions on nuclear receptor 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. Effect rating using induction of antioxidative enzymes was found in all active fractions. 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 multi-or single compounds using a combination of extraction, fractionation and bioassay has shown that 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, bioassaying 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 four-effect columns. The candidate peaks common in bioactive fractions are subjected to toxicitant 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: aminopropryl-, octadecyl-, pyrenyl ethyl and a pentfluorophenyl phase. A novel, downscaled antiAR-CALUX assay using low-volume exposure and chemical dilution procedures was applied for bioest 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-LCMS. Some of the bioactive peaks were effect directed. 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 (C48) and 7-ethylaminocoumarin (C51). The presence of the identified coumarin compounds was further evaluated in a higher trophic level using the spiggin-gsf 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 multiple surface waters, with the focus on aromatic amines and to apply a 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 total of 440 water samples were subjected to an online LVSPE machine 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 Rayom (BR) in six consecutive weeks. The mutagenicity of the BR extracts was evaluated using the strain TA98 and the samples 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-Exact 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: α-toluidine and 2-6-xylene, alkaldoids including the co-mutagen norharman and two of its isomers carboline and 5-carboline were revealed. Furthermore, 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-diaminophenazone 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, this study focused on the development of 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 (PSII) 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 these one herbicides, linuron, which occurred at 110 times the EQS concentration and which is not included in the WFD priority compounds list. In conclusion, algal photosynthesis bioassays focusing on aromatic amines and in particular mutagens, 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 process. Therefore, we classify the LCA with uncertainty 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 stakeholders 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 key factor to the decision making. 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. The relevent analysis scheme, e.g. cost-benefit analysis (CBA), risk assessment and life cycle assessment (LCA), 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.

406 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 requirement of information at each stage of the decision process is met to address uncertainty. The subsequent paper will outline this methodology 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 hierarchical 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 predict and reduce uncertainty in early design stage. By using decision making 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 on addressing the new approaches for improving eco-design within aerospace industry. In further 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.

407 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 circularity of plastic use and end-of-life 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 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.

408 Unilever’s framework for assessing impacts of bio-based raw materials

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Policy making should be supported by decision tools which analyse (i) caused & prevented damages and (ii) whether we have an adequate HA toolbox and techniques to extrapolate from single species laboratory tests to the field. There is a need to improve our understanding of the importance of scale changes. These include improving understanding of the leaching and biodiversity impacts of future raw material production and sourcing. To ensure the environmental gain and the economic success of policy decision, we looked into the decision analysis tools to enhance their assessment of environmental impacts. To 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, e.g. for different ingredients, is therefore made based on Unilever’s novel framework 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.

409 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 supporting tools which use similar goal and scope, functional unit and system boundaries. However, 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.

413 Downcycling vs Recycling – A conceptual proposal for addressing environmental and ecological variability when converting waste to product

B. van de Water, Leiden University

Increasing application of ecological knowledge should increase the scope for uncertainty in extrapolation from single species laboratory tests to the field. There are still challenges in making some test methods fit for purpose in hazard identification and regulatory conclusions on risk assessment and classification for higher-tier (e.g. reactivity) while the biokinetics (i.e. toxicokinetics) aspect is often missing. When the science justifications. When the science at organisms and sub-organism levels mostly adapt/mimic those from mammalian to organisms and sub-organism levels mostly adapt/mimic those from mammalian
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 analysing four methodological 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 determining high-quality recycling. Landfill tax, gate fees for the 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)**

410 New approaches in regulatory science  
D. Auteri, EFSA - European Food Safety Authority / Pesticides; S. Barmaz, EFSA; D. Auteri, EFSA - European Food Safety Authority / Pesticides

**The REACH Regulation** provides a high level of protection for human health and the environment. The regulator 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 not in adequate 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 bioinformatics (i.e. toxicokinetics) aspect is often missing. Improved read-across and WoE justifications for higher-tier (complex) endpoints often fit 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 (‘bioinformatics’) 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.

411 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. i.e. 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 for greater ecological and environmental relevance. Otherwise we are still mostly interested in 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 function production, could provide a broader ecological basis for identifying impacts on key ecological entities that in turn could inform HA. ii) Even though current environmental assessments are 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 late test methods such as the OECD Test No. 406, indicate that non-EC test methods 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.

412 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

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/minic those from mammalian toxicity 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.

413 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 adverse outcome. The model solutions will be hierarchically structured and based on the 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: i) 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.

414 Panel discussion
21st century Ecotoxicology and Human Toxicology: Applications and perspectives for the use of OMICS data (III)

415 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 exposures, gene expression, we need to characterize 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 urban and rural environments. 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.

416 Stress response pathways in neuro-behavioural and reproductive toxicity of graphene oxide in C. elegans: Integrative multi-OMICS approach
Y. Kim, University of Seoul / School of Environmental Engineering; C.P. Roca, 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 exposures, gene expression, we need to characterize 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 urban and rural environments. 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.

417 Molecular targets of narcosis and acetylcholine esterase inhibitors: Applications for chemical screening and identification using omics read-outs
E.K. Brockmeier, University of Liverpool / Institute of Integrative Biology; E. Butler, C. Rendell, Unilever / Safety and Environmental Assurance Centre SEAC;
S. Gutsell, Unilever / SEAC; F. Falciani, University of Liverpool / Institute of Integrative Biology
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 esterase inhibitors, the transcriptional responses showed a strong difference between the two groups. However, predictive tools like GALGO allow us 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.

418 The integrated use of omics technologies to understand mechanisms of Daphnia’s stress response to cyanobacteria
J. Asselman, Ghent University / Laboratory for Environmental Toxicology and Aquatic Ecology GhEnToxLab unit; D. De Coninck, Ghent University / Laboratory of Environmental Toxicology. Aquatic Ecology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; J. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; K. De Schamphelaere, Ghent University (UGent) / Applied Ecology and Environmental Biology
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 different Daphnia clones with different sensitivities to Microcystis. We used whole genome bisulfite sequencing to characterize the cytosine methylation in response to Microcystis stress. 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 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. There was significant enrichment of cytosines located in transcribed, including Microcystis-specific genes, 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.
421 Assessing the condition of forests exposed to climate change and nitrogen deposition by use of big monitoring data

W. Schröder, U. Nienicke, V. Deck, University of Vechta / Chair of Landscape Ecology; M. Jensen, 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 projected 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 through climate change. The projected changes are mainly driven by changes 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.

422 Dissentangling 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 concentrations 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 limiting river macroinvertebrate communities? 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 aquatic 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 geographical features and ecosystems, providing a broad basis for understanding how physico-chemical conditions limit global freshwater biodiversity.

423 Compilation and analysis of monitoring data from eutrophied lakes across Europe: experiences from EU projects

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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 emerging pollutants, but to a lesser degree on 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.

424 State-of-art and SWOT-analysis of existing fresh water monitoring approaches
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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 predicting the ecotoxic 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, essentially 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)

425 Impact of cleaning procedures and type of materials on the measure of 20 bisphenols in surface water samples
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These pharmaceuticals and metabolites have been detected in ng L-1 concentrations considering pseudo-first order kinetics, below 30 s at pH 7) than chloramination (monochloramine under laboratory conditions. Chlorination showed to be more effective in phenoxy- and phenazine-type pharmaceuticals, but for 9 out of 20 (essentially Bisphenol A and bisphenol BP). This demonstrates 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 far as the enantiomeric enrichment factor is known.

427 Chlorination and chloramination of phenazo-type drugs and metabolites
B. Sieira, University of Santiago de Compostela; R. Rodil, University of Santiago de Compostela; J. Quintana, R. Cela, University of Santiago de Compostela

Phenazo-type pharmaceuticals, such as aminopyrine, metamizole, phenazi (Phe) and propyphenazone (PrPhe), are widely used analgesics. Aminopyrine and metamizole are extensively metabolized in the human organism to aminooxpyrine (AA), 4-aminooxpyrine (AAP) and 4-formyl-aminoxyprine (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 phenoxy- and phenazine-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 µg mL-1. pH values ranging from 5.7 to 8.3 and bromide concentration ranging from 0 to 200 mg 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. Pharmacokin., 28, 216–234. [2] Agüende, J.A.G., Martinez, C., Benítez, J., 1995. Metabolism of aminopyrine and derivatives in man: in vivo study of monomorphic and polymorphic metabolic pathways. Xenobiotaica 25, 417-427. [3] Wiegel, S., Auling, A., Brockmeyer, R., Harns, H., Löfker, J., Reinecke, H., Schmidt, R., Stachel, B., von Tümpling, W., Wanke, A., 2004. Pharmacuticals 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.

428 Technical and economic assessment of real-time control strategies for micropollutant removal by ozonation at pilot-scale
M.E. Chys, W.T. Audenaert, Ghent University; A. Marchi, M. Weemaes, Aquafin; M. Van Langenhouve, Ghent University / Sustainable Organic Chemistry and Technology Research Group EnVOC; S. Van Hulle, Ghent University / Campus Kotrijr

Oil Sands: integrate natural processes in hazard assessment
S. CHIRON, M. Brienza, IRD

Recent developments in modelling the environmental fate of substances in oil sands and in the surrounding environment have allowed for assessing and mitigating the environmental impact of industry and waste disposal. This presentation will focus on recent and ongoing models for simulating contaminant release from oil sands and their interaction with the environment. The role of natural processes such as biodegradation and transport is particularly emphasized.

429 Enantiomeric fractionation for quantitative assessment of anaroeic biodegradation rates: The case of climbazole
S. CHIRON, M. Brienza, IRD

An efficient chiral liquid chromatography - high resolution - mass spectrometry method has been developed for the determination of climbazole (CBZ) enantiomers in wastewater / sludge. The optimized analytical method has been validated with good quality parameters including resolution > 1.1 and method quantification limits down to the ng L-1 range. On the basis of this newly developed analytical method, the stereoselectivity of CBZ degradation in water and its main metabolic products was estimated. Diastelective degradation was exclusively observed under biotic conditions, confirming the specificity of enantiomeric fraction variations to biodegradation processes. CBZ was always biotransformed into 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 (R² > 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 far as the enantiomeric enrichment factor is known.

Acknowledgements

C. riparius

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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 (DUVÄ254) to MP removal, has been recognized and novel empirical models have been suggested based on lab-scale experiments. Reliable surrogates 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 oozie 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 DUVÄ254 was compared with two more conventional alternatives: flow-based (i.e. a fixed O2 dose expressed as mg O2 L−1) and load-based (i.e. a fixed O2: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 O2:DOC ratio strategy resulted in more or less similar costs as the DUVÄ254 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 DUVÄ254 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.

429
Poster spotlight: WE091, WE092, WE093, WE094

Environmental consequences of oil and gas extraction and transport

430 Differences in exposure media profile and toxicity following preparation of water-accommodated fractions along an energy continuum
B.A. Stubbsfield, B. de Jourdan, Oregon State University / Environmental and Molecular Toxicology

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, 200 L/min vortext), Low Energy (LE, 40 L/min vortex), Intermediate Energy (IE, 60 L/min vortext), 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 Cypripodan variegates) and 48-h static (American shad (Alosa sapidus) 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.

431 Development of a Generic Exposure Scenario for substances used in high volume hydraulic fracturing fluid products under the REACH regulation
N. Vallotton, Dow Europe GmbH / Toxicology Environmental Research and Consulting; J. Worden, Shell International / Shell Health -Risk Science Team; M. Leon Paumen, ExxonMobil Biomedical Sciences / ExxonMobil Biomedical Sciences TES Division; J. Getliff, Chevron North Sea Limited; S. Peter, BASF SE; N. Robinson, NIKAM Consulting Limited

The European Commission Recommendation (2014/70/EU) on minimum precautions 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 methods or more relevant environmental labels (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 recent scientific data and assumes 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 support 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.

432 Oil Sands: Integrate natural processes in hazard assessment
D. Nunes Cardoso, CESAM, University of Aveiro / Department of Biology and CESAM; J. Pestana, CESAM & University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology & CESAM; 3810-193 Aveiro; F. Wrona, Alberta Environment & Parks / Science Division; S. Loureiro, Universidade de Aveiro / Biology

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-organic 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 (i.e., 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 proportion bitumen content due to the differences in the specific geological formations in each river basin. The toxicity 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 contaminated soils 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 emphasises the need to better discriminate natural processes from anthropogenic sources (mining related activities) of surface and groundwater contamination in oil sands areas.

433 Fate and effects of oil in ice
M.G. Smij, Shell Global Solutions Int.; A. Basseres, TOTAL S.A. / Environment; J. Mullin, Joseph Mullin Consulting LLC

The Arctic Oil Spill Response Technology Joint Industry Programme had the goal to develop and demonstrate 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 along with available and applicable methods for following oil spills after the event. This 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 mesocosm were installed for high volume of oil at the NAMSCOT in January 2015 and remained in place until July 2015. Oil was introduced into two
Environmental risk assessment in time and space - To boldly go where no man has gone before

Molecular Landscape, the integration of adverse outcome pathways into a regional scale pesticide driven risk assessment for Chinook salmon in Puget Sound watersheds.

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 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, the ERA in pesticides has three main advantages: (1), local environmental conditions can be considered for risk assessment decisions; (2), the expected impact for the environment and ecosystem services due to risk management 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 fludioxonil 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 km² 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 population following stress events. In addition, generic ERA was performed by linking FOCUS TOXSWA simulations for the representative Dutch ditch scenario to the same TK/TD and population modelling.

Individual-based modelling as a population-level risk-assessment tool in the identification of emerging pollutants in European catchments

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 approach we used the chemical’s impact on projected population growth rate 1, 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 E50 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 Asellus aquaticus, evaluating WFD priority substances. The impact was quantified by the ratio of daily population growth rates under exposed and reference conditions (D/E<sub>ref</sub>). Results comprise the relative population growth rates for >3,000 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] Lindm, 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.

Integration of aquatic exposure and effect models at landscape scales: an example case study and some conceptual considerations

Current environmental risk assessment (ERA) of pesticides in aquatic systems is rather generic. This is mainly on the effect side of risk assessment generic threshold

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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 built upon an ecosystem approach for decision-making that incorporates a variety of management options, can point to the data requirements, defines monitoring goals, and can include externalities such as climate change.
349 Influence of local habitat connectivity and isolation on plant community resilience
J. Reeg, University of Potsdam / Plant Ecology and Nature Conservation; S. Heine, Bayer AG / Section IV Environmental Risk Assessment of Pharmaceuticals; C. Mihan, Bayer CropScience AG / Ecotoxicology; T. Preuss, Bayer AG / Environmental Science; M. Görfinko, Bayer CropScience AG / Environmental Science; E. Mahncke, Bayer CropScience AG / Environmental Science.
Small 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 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)

440 Associations of nanoplastic with freshwater algal traced using a novel florescence assay
C. Liddle, Heriot Watt University / School of Life Sciences; C.R. Tyler, University of Exeter / Biosciences College of Life and Environmental Sciences; T.S. Galloway, University of Exeter / Department of Biosciences
 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 has focused on their role in the delivery of deleterious chemicals, like endocrine disruptors and other chemicals. In this study, we used the fluorescent nanocomposite Amilm to examine the effects of polyethylene microplastics on freshwater benthic invertebrates. The results showed that the uptake of polyethylene microplastics increased with the concentration of the pollutants used. Furthermore, the majority of the species studied were 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 polyethylene microplastics has been found to be strongly correlated with the presence of microplastics in the environment. These results suggest that microplastics can act as a source of toxic substances, and this may have important implications for the conservation of freshwater ecosystems.

Chronic effects of polystyrene microplastics on freshwater benthic macroinvertebrates
P. Redondo Hasselberg, D. Fahaludin, E. Peeters, A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management
Understanding the interaction between microplastics 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 microplastics ingested and how the sensitivity of certain species can be affected by these particles. 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 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 measured the effects of polystyrene microplastics on the survival and growth of the amphipod Gammarus pulex and the worm Lumbriculus variegatus.

441 The presence of microplastics affects toxicity of the pesticides deltamethrin and dimethoate to Daphnia magna differently, based on differing hydrophobicities
A.A. Harm, Centre for Ecology and Hydrology; E. Lahive, Centre for Ecology & Hydrology (NERC); P.M. Van Bodegom, CML Leiden University / Institute of Environmental Science CML; R. Heutink, University of Leiden / Institute of Environmental Sciences CML; D. Spurgeon, J. Baas, Centre for Ecology & Hydrology; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology; M.G. Vijver, CML Leiden University / Conservation Biology
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 study were the 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 (1 mm 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 deltamethrin and dimethoate, to understand how the presence of microplastics may affect toxicity of pesticides based on differences in binding capacity. We hypothesised that the toxicity of deltamethrin will be more significantly influenced by the presence of microplastics due to its high hydrophobicity.
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 the highest 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.

443 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 < 5 mm. 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 endocrine-disrupting compounds. Although numerous studies have shown that 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 RTWL-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.

444 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 nanoparticles (< 100 nm). Generally they have a large surface area and distinctive characteristics. In this study, we carried out the individual toxicity tests on four ecosystems (Chlamydomonas reinhardtii, Daphnia magna, Oryzias sinensis and Zacco temminckii) in freshwater ecosystems and trophic transfer test on these species using green fluorescent nanoparticles. First, we exposed nanoparticles 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 viability (O. sinensis) and total cholesterol of fish. Fish swim the limited areas when they were exposed to nanoparticles 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 nanoparticles was visually confirmed in the gastrointestinal tract and feces of test species. In conclusion, nanoparticles 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 Foundation of Korea (NRF) funded by the Ministry of Science, ICT and future planning (2016R1A2B3010445).

445 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 carbon and nitrogen 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 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 bryophytes, 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.
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 Remediation of Contaminated Soils and Land (GARSA) project at the University 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 the environmental and human health risk results obtained in the pilot study conducted in the rehabilitation efforts of Cartagena-La Union 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 pond 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 (phytosorption). 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.

448 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 Mariginaec, 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 10ha 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, geochemistry, 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 (NH3>500 ppm, HCN>30 ppm, H2>1 500 ppm). In regards of mineralogy, substantial heterogeneity was apparent both vertically and horizontally, 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 geochemical variable that could define geochemical activity within the pile. If CaO/MgO was < 5 then a strong likelihood existed for higher temperatures and gaseous (NH3, 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.

449 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 accumulation over time. Bauxite tailings 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, phytoremediation (plant uptake) has emerged as a potential, 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 directly interact with them. This will help to support the decision-making of the remediated sites.

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

450 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 to combat climate change and reduce environmental and 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.

451 Life Cycle Sustainability Assessment for photovoltaic panels using sofa E. Eisfeldt, C. Rodriguez, GreenDelta GmbH; A. Ciroth, GreenDelta
“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.2 database, called sofa, was developed to calculate impacts of life cycles. 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 PSIILCA 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 elemental 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 documents the calculation on the process level. As a result, sofa 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 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.

452 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 LCAs methodology in different products and sectors. The methodology employed in this work is based on the LCA + Analysis approach and has been applied to evaluate the sustainability of the emerging Concentrated Solar Power technology (CSPTec). The integration of 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 impacts of the CSPTec (HYSOL BIO for low-cost biomass and HYSOL NG for operation with natural gas). In the economic area, a Life Cycle Cost Analysis and a Multi-regional Input Output Analysis were performed in order to estimate the life cycle costs and economic effects of the HYSOL BIO, HYSOL NG 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 biomass enabled 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.

453 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 innovations. A key issue in the use of models is 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 LCA 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 identify 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 level 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 Impact2000+ 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 energy production and the largest 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.

454 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 project 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 and the mix of the energy system 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.

455 Regulatory Best Practices for Assessment of Endocrine Active Substances

During the last decades, the regulatory communities in different parts of the world have been debating how EDCs 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 regulations 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.
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 facilitates regulatory enforcement for top toxicocasts, 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 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-throughput 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 PBTGs using PBSTP standards, examples of assay types where PBSTP assays 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 PBSTPs.

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Applying high-throughput screening data to define key events for applied 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 key events that lead to adverse effects of regulatory concern. For example, several endocrine modes of action (i.e., altered estrogen receptor agonism, thyroid 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 key events for endocrine and non-endocrine toxicity pathways leading to impaired vitellogenesis. AOP 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-Tetrachlorovinphos, 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 an isocyanate (endocrine dysregulation) and a hydrocarbon (endocrine dysregulation), which led to the hypothesis that these compounds had similar non-endocrine toxicity pathways and that the ToxPi model could be used to predict non-endocrine toxicities.

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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, 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 exhibit ecological effects. 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 substance if it was found to be toxic. 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 and female pinnae counts at the 23 μg/L level were consistent with apparent growth effects at this level and were attributed to general toxicity at 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 pinnae were not endocrine mediated. The results of this study is in agreement with the other lines of evidence 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 in the hypothesis-driven weight of evidence evaluation of triclosan, which is a fungicide. 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 toxicities
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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 recyclates. 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 heavy metal 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 17 PAHs varied from 10 to 40 mg/kg. Pyrene was the dominating PAH with levels up to 1.4 mg/kg. The 7-day "carcinogenic" potential of these 17 PAHs were 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 74 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 cytochrome P450
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.

461 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 plastic food containers are consumed by the public and can end up in the environment. The increasing recycling of food contact articles (FCAs), like conveyer 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 food. Preventing food contamination is a relevant 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 173 known COCs can be legally used in FCMS, and some of these substances migrate into foods. Therefore, FCAs are relevant exposure sources 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.

462 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 driving forces toward a circular economy. Among recyclable materials, wood waste is a relevant source for recycled materials. However, the recycling of wood waste is complicated by their complex composition, including a mixture of organic and inorganic substances. Therefore, it is important to assess the presence of impurities in wood waste collected at recycling centers and meant for recycling. For economy perspective, cascading of material uses should be applied, and wood waste is a relevant 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.

463 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 livelihoods for the poor, but is nevertheless characterized by poor 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 these products (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 collected in Delhi to test both the method and the feasibility of the 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 to due 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.

464 Towards 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 within the waste hierarchy (technical 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 collected in Delhi to test both the method and the feasibility of the 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 to due 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.

465 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 use 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 recycling. 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 material. Environmental contaminants: wooden items 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 be sufficient to ensure that wood waste collected at recycling centers and meant for recycling is a sustainable solution for HBCD containing PS foam via a dissolving technique with bromine recovery.
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 Coolsolv® 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)

465 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 to 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 high throughput screening (HTS) strategies that 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.

466 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) guidance1 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 larval 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 larval toxicity tests for pharmaceuticals were discussed as well as 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 (EMA), London, UK. EMEA/CHMP/SWP/4447/01. 6 June 2006. 2 Mørmord CTA, Kase R, Korkarc M, Agebrand M. 2015. CRED: criteria for reporting and evaluating ecotoxicity data. Environ. Toxicol. Chem. 35:1297-1309

467 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 Leptinotarsa decemlineata (Trichoptera; crenatibellatae) or Leptinotarsa decemlineata (Trichoptera; crenatibellatae). There is a close relationship between 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 toxicity of trichopteran Cry1Ab toxin with a food-spiking method. Black alder (Alnus glutinosa) leaves were 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., Chaetoptygex spec.) were conducted. The larvae were fed with spiked leaf discs in different concentrations over 6 or 12 weeks (10 rep.). 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 bioassay and mortality was recorded in order to verify the food-spiking method. The Cry1Ab toxin concentrations of the spiked leaves were measured on the 7th day using a one-linkage ELISA (ELISA)/(Agdia, Elkhart, USA) and revealed that the spiking method is able to prepare different toxin concentrations. The Chaetoptygex 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 cadyslfy experiments showed that Cry1Ab toxin might have slight influence if exposure happens as part of their food.
No changes were registered in vpg 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 living metabolism impairing bile acid synthesis, detoxification and multipotent endocrine effects. This encouraged us to develop a novel test method for assessing fish xenobiotic toxicity. This was sponsored by the European Community under contract INCO-DEV (No G5RD-CT-2001-00248-09) and was assessed by a multinational expert panel. Funded by: Spanish MINECO and EU-FEDER (AGL2015-63936-R; CTM2014-56628-C3-1-R). Basque regional government (IT810-13; UPV/EHU (UIF 11/37).

471 Thyroid hormone disrupting effects in zebrafish exposed to compounds found in and for the environment

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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 sample 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 therefore presented. In addition, it is shown that artificial endpoints related to the thyroid glands are used, as well as artificial endpoints related to the presence of thyroid disrupting effects are measured. 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, hatch, heartbeat, swimming behaviour and swim bladder inflation was monitored. 25 compounds were selected based on their detection in cat, fetal and maternal serum samples and dust extracts. Artificial endpoints 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 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 exposure to indoor related chemicals and their thyroidogenic effects in mixtures. The project is aimed to feed in scientific results for improved management of EDCs.

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

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G. G. 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 currently used test 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 ≥0.127 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 transgenerational. Nevertheless, N. furzeri is a valuable tool for preventing excessive or unproductive toxicity testing. As an example, we investigated the genotoxic and ecotoxicological effects of copper in zebrafish (Danio rerio). We expose zebrafish for 10 days, followed by a recovery period in clean water for 10 days. The fish were then exposed to copper for 10 days and after this period, we monitor the effects of copper on gene and protein expression.

Fish model species in human and environmental toxicology (I)

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

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Xenobiotics are chemicals that are present in aquatic habitats and may feminize fish populations. Chemicals such as bisphenol A, alkylphenols and synthetic hormones are dispersed 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 exposure to a mixture of four well known xenoestrogens in the hepatic metabolism of juvenile fish. European sea bass (Dicentrarchus labrax) juveniles were 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 (fmr), liver-X-receptor (lxr) and hmg-CoA reductase were amplified and sequenced for qPCR analysis. Likewise, genes involved in the detoxification metabolism pathway such as cyp1a, cyp3a, udp and abch11 were investigated as well as vitellogenin (vg1), 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 cyp7a1, fmr, lxr, cyp3a and udp were down-regulated, whereas hmg-Coar, cyp3a and abch11 were not altered. After 7 days of exposure all these genes as well as cyp3a and udp were upregulated.
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.

473 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 currently available method for validation of different toxic effects in testing an organism, 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 feedings 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.

474 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, owing 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) 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, estrogenicity 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 human, 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

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

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Establishing a robust in vitro exposure system is still very challenging, particularly for medium 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 hydrophobic binding nor for low systemic concentrations 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 be applicable to other toxicants and exposure conditions, with a significant difference between EC90s 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 L15ex medium in 24-wall plates after 24h exposures and for several other experimental set-ups.

476 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 ($K_{br}$), 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.

477 The chemical exposure toxicity space (CETS) model: expanding its application to non-fish organisms including Hyla azteca and Lumbirculus variegatus.

A. Kramer, Queens University; D. Mackay, Trent University / Chemistry; J. Parnis, Trent University / Cemistry; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; L.S. McCarty, L.S. McCarty Scientific Research & Consulting; D.E. Powell, Dow Corning Corporation / Environmental Sciences

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 puppines 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, *Hyla azteca* and *Lumbirculus variegatus* using published bioassay test data [1]. A full description of the model is currently in press and has been accepted for publication.
glutathione dynamics in the organogenesis stage rat embryo. The CETS model will be used to reduce animal usage in toxicity testing. Future developments will provide 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 concentrations and activities. For several assays it was 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 sample. Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) using electrospray ionization (ESI) at multiple reaction monitoring (MRM) mode, and analyses were quantified with isotope-dilution techniques. PAEs and five basic PCPs were separated on an Ascentis Express 5 column (30 x 2.1 mm, 2 μm) with mobile phases of 5 mM ammonium acetate (aq) and 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 313.9 > 148.9. Trace amount of DEHP (191 pg/mL), diisononyl phthalate (DINP, 229 pg/mL), and dioctyl phthalate (DOP; 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 x 2.1 mm, 1.5 μm) with 0.04% acetic acid and methanol for gradient elution. Uses of methanol and acetone eluted analytes better from SilicaBond 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 60-66%). 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.

481 Determination of opioid analgesics and their metabolites in municipal wastewaters by liquid chromatography-tandem mass spectrometry of pharmaceuticals in arctic and sub-arctic environments. In Nuuk, the capital and largest town of Greenland, increasing attention is paid to the pollution levels of selected environmental contaminants and their mixtures. Perfluorinated compounds (PFASs), such as perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA), and perfluorobutane sulfonate (PFBS), have been found ubiquitously 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 eluent was evaporated to a small volume and reconstituted 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 313.9 > 148.9. Trace amount of DEHP (191 pg/mL), diisononyl phthalate (DINP, 229 pg/mL), and dioctyl phthalate (DOP; 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 x 2.1 mm, 1.5 μm) with 0.04% acetic acid and methanol for gradient elution. Uses of methanol and acetone eluted analytes better from SilicaBond 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 60-66%). 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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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 analogues from aqueous samples and suspended solids were performed employing solid-phase extraction (SPE) based on mixed extraction–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 determined by using Oasis MCX cartridges (recoveries 93-91%). 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-acetyl morphine 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 analogues, LC-MS/MS, wastewaters

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Tracing micropollutants in raw and drinking water in Sweden
R. Tröger, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment; L. Åhlund, Swedish University of Agricultural Sciences / Water Science and Assessment; V. Frank, L. Gill, C. Wahlberg, P. Klockner, D. Kothawalla, Swedish University of Agricultural Sciences; S.J. Köhler, K. Wiberg, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment

A multi-residue method was developed for detection of a wide range of micropollutants at sub-µg 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 (DWT), 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 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 (fluoxazolae, ibrostan, carbamazepine, naproxen, paracetamol, and venlafaxine) and in addition PFASs (PFBS, PFHxS, PFDA, 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 described a suspect screening method that uses UHPLC-QTMS chromatography, 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 DWTs.

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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 periphery and three receiving seawaters in Nuuk, Greenland, was 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 their isotopic 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, guarantees 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 0.0-1.4%, respectively. 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 μg 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 μg 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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Tranhyretin-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 capacity 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 experimentially 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, usually determined in the first meter of the house, a total exposure to the in vitro IC50 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 house-dust 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 response of zebrafish and human-based estrogenicity bioassays to selected environmental contaminants and their mixtures
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INERIS / Ecotoxicology Unit

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 zERα, ZELHβ2 cells expressing zERβ2) 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 culture condition (metformin). 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 selectively active either on human or 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 potency 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.

487 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 spread over several days. It is therefore not only the dose that makes the poison but also the exposure sequence.

488 A non-toxic aquatic environment: a Paracellic 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 Paracellic view, everything is toxic, but it is the concentration that would make the environment toxic 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 mixture and sensitivity models with field observations 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 management of mixture-exposures, with latitude for defining 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’. Sensitivity assessments of the individual compound 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 of 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.

489 Poster spotlight: TH128, TH129, TH130, TH132

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

490 High-selenium lentil 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 1990 the 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 №372, December 2012) and greater than the Bangladesh limit of 50 ppb. Selenium (Se), an important nutritional trace element can antagonize As, so efforts to relieve arsenic effects (chronic arsenic poisoning) have included daily treatment with Se pills. This would impose 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 ecosystem. 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.

491 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 internal exposure to iAs (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 were compared with biomonitoring data from a Belgian monitoring campaign (Flemish Government 2008). Van Holderbeke et al., 2009b. In order to verify the model predictions iAs exposure and iAs body burden at the end of 2002, MERLIN-Expo was used to carry out a new, 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.

492 The DustEx model for calculating human exposure to semi volatile 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 by ingestion 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 organics (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.

493 Internal dosimetry metrics for risk assessment of endocrine disruptors - the case of bisphenol A

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A major outcome of the FP7 project for H2020’s 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 PBPK 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 PBPK model, two different exposure metrics were used: The temporary Tolerable Daily Intake (t-TDI) of 4 μg/kg body weight per day 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; in vivo, 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 glucuronyltransferases. The results indicate that taking into account these 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.

494 Poster spotlight: TH153, TH154, TH155, TH157

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

495 Mechanical fragmentation of ingested microplastics by Antarctic krill

Dancing, Griffith University; S. Kawaguchi, Australian Antarctic Division / Australian Government Department of the Environment; C.K. King, Australian Antarctic Division; W. Huston, University of Technology Sydney / School of Life Sciences; K. Townsend, The University of Queensland / University of Queensland; R. King, Australian Antarctic Division / Australian Government Department of the Environment; S. Bengtsson Nash, Griffith University / Southern Ocean Persistent Organic Pollutants Program

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.

496 Adverse effects of microplastics and oxidative stress-induced MAPK/Nrf2 pathway-mediated defense mechanisms in the marine copepod Paracyclops nana

H. Kang, Sungkyunkwan University; C. Jeong, Hanyang Univ.; J. Lee, Sungkyunkwan University

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 Paracyclops 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 driven pathway for microplastic exposure at the 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.

497 Rapid filtering and ingestion of microplastics by marine mussels under ecologically-relevant scenarios

C. Goncalves, M. Martins, MARINE Marine and Environmental Sciences Centre / Departamento de Ciências e Engenharia do Ambiente; P. Sobral, MARINE Marine and Environmental Sciences Centre; P.M. Costa, M.H. Costa, MARINE Marine and Environmental Sciences Centre / Departamento de Ciências e Engenharia do Ambiente

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 microplastics in oceans, with potential consequences to the biota. In order to evaluate the effects of plastic
levels are higher in the control of each treatment in comparison to MPI and MPV.

The ingestion of polyethylene microplastic particles from toothpaste is particularly relevant to realistically long exposures to micromaterials that even such inflammatory-like responses, at least during the time of the bioassays and under the digestive tract. Here, they accumulate and eventually generate moderate effects on fish and, potentially, on human health.

548 Ingestion in blue tautog (Mytilus galloprovincialis) caused by the ingestion of polyethylene microplastic particles from toothpaste

J. Næsland, Norwegian Institute for Water Research NIVA; M. Blazquez, CSIC Spanish National Research Council / Marine Renewable Resources; S. Brooks, NIVA / Ecotoxicology and Risk Assessment; K.V. Thomas, Norwegian Institute for Water Research NIVA

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 galloprovincialis) 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 Øss RDF 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 mantle.

549 Gene expression in liver of European sea bass (Dicentrarchus labrax) experimentally exposed to PVC microplastics

C. Beier, University of Göttingen / Department of Environmental Physical Earth and Environmental Sciences; E. de Rysky, University of Siena / Department of Environmental, Earth and Physical Sciences; C. Pedra, ISPRRA; G. Maricichio, IAMC, Institute for Coastal Marine Environment; L. Genovese, IAMC, Institute for Coastal Marine Environment CNR; U.O.S. Messina; F. Gai, Institute of Science of Food Production, CNR; T. Romeo, ISPRA-Institute for Environmental Protection and Research / Laboratory of Milazzo; M. Fossi, University of Siena / Department of Environmental Earth and Physical Sciences

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 environment, especially in feed. Time Point (TP) and 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 (MP) 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 PPARα (Perilipin A) and its receptor (PPARγ), which mediate the responses to several chemicals including plasticizers; the Estrogen Receptor alpha (ERα), a 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 MP) 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.

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

500 Un-characterized elemental 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 to a 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 (8% as mass). The high (24% as mass) occurrence of high (higher than 24%) un-characterized characterizations points to the high potential of flows to 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 LCA phase should always be done to support interpretation of results. Indeed, the characterization of the inventory into potential environmental impacts may not occur if some of the elementary flows are not covered by the characterization models. Toxicity impact categories in LCA and the Product Environmental Footprint (PEF) are often the dominating impact (after normalization) and metals are often identified as the most contributing elementary flows (ELFLs).

501 Getting the chemicals right: Gaps and opportunities in addressing inorganic 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 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 LCA toxicity characterization models, the economic and environmental relevance of inorganic chemicals, and an outline of possible ways towards incorporating inorganic chemicals in LCA toxicity characterization. The analysis of existing LCA studies 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 the specific substance groups often result in large differences to existing models and methods. Toxicity impact categories in LCA 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, the new test methods show that the existing LCA model USEtox cannot be applied to inorganic substances without further modification towards including specific reaction- and process-kinetics. 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.
A trophic chain-based approach for ranking chemicals in LCA

E. Croonen, European Commission - Joint Research Centre / Directorate D - Sustainable resources, Bioeconomy Unit; E. Saouter, EU Commission JRC / Sustainable Assessment Unit; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

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 then be used, such as in USEtox, for ranking chemicals. The study aims at presenting the methodology and discussing the potential added value of using few species. Current results are to be considered for illustrative purpose only.

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

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Metal contaminants are among the most challenging components 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 kfc 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 kfc 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 IMPACT2000 and/or USEtox. For each field soil sample, two BFsc (BFsed,Fc based on field data and BFsed,AM 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 BFsc 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 Canada. New CFs were validated on soil samples and indicate so far 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.

504 Roofing 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 EA face many issues to account for the fate and 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 operator 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 experts, in order to “harmonize” terminologies 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 (available through the AIEL); 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 standardized methods and their use in regulatory frameworks

505 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 legal, ethical, 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 responses under test. 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 disconnect is Evaded-Based (EB) methods and specific test methods, as well as the need for a general method 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 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.

506 Species Sensitivity Distributions with Censored Values

J.W. Green, DuPont / Dynamic Simulation, Control, and Optimization

Species Sensitivity Distributions (SSDs) are increasingly used 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 is 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 HCS 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. 2013) and others are using the log-normal distribution. 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 disconnect is Evaded-Based (EB) methods and specific test methods, as well as the need for a general method 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 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.
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.

507 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 components 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/m². Toxicity was observed in all tests and particularly two products showed elevated toxicity. For Product 1, >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.

508 Are current algae toxicity tests suitable for the evaluation of carbon-based nanomaterials?
J. Farkas, SINTEF Materials and Chemistry; A. Booth, SINTEF Ocean / Environmental Technology

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 10 μL of a 200 μM CNM solution. 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 CNMs is a challenging task and this study emphasizes the need for further research towards the development and establishment of standard TG which are suitable for all MNM types.

509 Poster spotlight: TH232, TH233

Global Horizon Scanning Project - open session

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

511 Research needs for high-throughput assessment of individual contaminants and their mixtures, both historical and emerging, and sustainable molecular design and alternative analysis of chemical products
B.W. Brooks, Baylor University / Department of Environmental Science

512 Prioritisation and detection of existing and emerging environmental contaminants
A. Boxall, University of York / Environment Department

513 Predicting stressor effects on ecosystems and their services in a changing world: implications for risk communication, risk management and restoration
L. Malby, The University of Sheffield / Dpt. of Animal & Plant Sciences

514 Discussion on way forward

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

515 The effects of DEHP, a potential endocrine disruptor, on an insect, the Egyptian cotton leafworm
A. Ayales, University Pierre et Marie Curie / Institute of Ecology and Environmental Sciences; I. Boulogne, Université de Rouen Normandie / UPRES-EA 4358 GlycoMev; A. Maria, Universite Pierre et Marie Curie / Institute of Ecology and Environmental Sciences; D. Renault, Université des Rennes / UMR CNRS ECOgesp, F. Bozzolani, Université Pierre et Marie Curie / Institute of Ecology and Environmental Sciences; M. Mathéhac, Université Pierre et Marie Curie / Department of Sensory Ecology; D. SIAUSSAT, Institute of Ecology and Environmental Sciences / Institute of Ecology and Environmental Sciences; D. Si (2-ethylhexyl) -phthalate (DEHP) 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 and could be potentially disrupted by DEHP. Besides, we investigated the potential modifications in the hemolymphatic ecdysoidlte larvate in larvae and adult males, using Enzyme Immuno Assay (EIA). 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 40ng 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 ecdysoidlte 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. ECHA - European Chemical Agency. 2008. European Union Risk Assessment Report –

516 Biochemical and transcriptional responses in the freshwater crayfish Procambarus clarkii exposed to pharmaceutical mixtures. C. Trombini, CSIC Spanish National Research Council ICMAN / ECOLOGY AND COASTAL MANAGEMENT; M. Hampil, CACTYMAR University of Cadiz; J. Blanco Moreno, CSIC Spanish National Research Council ICMAN / ECOLOGY AND COASTAL MANAGEMENT

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 fluoxetine (FL) are broad-spectrum antibiotics of the fluoroquinolones class. Fluoroquinolones are a highly produced class of antibiotics 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, Procambarus 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 θ), toll-like receptors (PTC-Toll) and inflammatory (collagen alpha chain-like protein CoLa) processes and neurotransmission modulation (neurpetide precursor protein NP, myosupressin-like neurpetide precursor MSNP, syntaphase-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.

517 BDE-47 induces oxidative stress, activates MAPK signalling pathway, and elevates de novo lipogenesis in the copepod Paracypselina nana M. Leq 1, Lee, University of Oslo, Norway

Bis(2-ethylhexyl) 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 impact 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 Paracypselina 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 C. magnum pathways. Activated MAPK pathways, in turn, induced signal molecules that bind with the transcription factors (TFs) responsible for lipogenesis to ECR, SREBP. CYP19/2 overexpression, stress stimulated the expression 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, 75-DES, 79-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.

518 Mechanisms of action of compounds that enhance storage lipid accumulation in Daphnia magna. C. Barata, CSIC - Environmental Chemistry; R. Jordão, CESAM & University of Aveiro / Biology; B. Campos, B. Pina, R. Tauler, IDAEE-CSIC / Environmental Chemistry; A. M. Soares, Universidade de Aveiro / Department of Biology & CESAM, 3810-193 Aveiro

Accumulation of storage lipids in the crustacean Daphnia magna can be altered by a number of exogenous and endogenous compounds, like 20- hydroxycycodexyn (naturally ligand of the ecysode receptor, ECR), methyl farnesate, pyrroproxin (agonist of the methyl farnesate receptor, MR) and tributyltin (agonist of the retinol 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 MR. 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 MR signaling pathways, either individually or in combination. RXR, Ecr and MR agonists increased storage lipid accumulation, whereas fenarimol and testosterone were anti-inflammatory (collagen alpha chain-like protein CoLa) processes and inflammatory (collagen alpha chain-like protein CoLa) processes and inflammatory. However, both fenarimol and fenarimol have been found to exhibit the same effects on the pro-inflammatory cytokine and number of larval instars, larval weight and food consumption) and the sex ratio of D. magna. These findings also provide evidence that dietary compounds can influence the establishment and development of natural populations of Daphnia magna in aquatic environments.

D-counter: Automatic Organism Counting and Characterization in Ecotoxicology assays S. Abreu, University of Aveiro / Dep. Biology & CESAM; F. Baptista, University of Aveiro; F. Jesus, University of Aveiro & CESAM / Department of Biology; A. M. Soares, Universidade de Aveiro / Department of Biology & CESAM, 3810-193 Aveiro; R.E. Martins, M. Oliveira e Silva, University of Aveiro / DETI EEETA

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 will present the first evidence of an 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 (0-210 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, r²=adjusted=0.990, F= 289.696, p< 0.0001). Regarding the life table experiment, no significant differences were observed between the counts of both technicians (Mann-Whitney U Test n=13512.000, n=136, z=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 Test n=36868.000, T= 13674.000, n=136, p= 0.755). The obtained regression (n=136, r²=adjusted=0.920, F= 1588.1205, p< 0.0001), corroborates that manual and D-counter counts are not statistically different. Overall results show the lack of significant differences between the manual and the D-counter estimated values with both BL and number of offspring and an EcR antagonist, respectively tool to be applied in ecotoxicology assays involving Daphnia sp, or other aquatic organisms such as Artemia sp. Preliminary tests also showed its application in Danio rerio eggs differentiation and counting.

Fish model species in human and environmental toxicology (II) 520 Toxicity and neurotoxicity profiling of European sediments samples with Daphnia in vivo embryos: A new multiparametric screening tool R. Massel, Helmholtz Centre for Environmental Research UFZ, Scholz, Helmholtz Centre for Environmental Research / Department of Bioanalytical Ecotoxicology; M. Krauss, Helmholtz centre for environmental research - UFZ / Effect Directed Analysis; T. Schulze, Helmholtz centre for environmental research - UFZ / Institute for Environmental Sciences; H. von Rheinach, University of Veterinary Medicine Vienna / Department of Systemic Ecology; W. Brack, Helmholtz Centre for...
to the two lowest concentrations, suggesting increased neuronal pathology. 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 m9 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.

523 Bioaccumulation and biological effects of tritiated water using zebrafish (Danio rerio) embry-o-larval model. To reach this objective, zebrafish eggs were exposed at two HTO concentrations (1.22×10^4 and 2.12×10^5 Bq mL^-1); fish were sampled at egg stage and larva stage. The first stage aimed at assessing HTO bioaccumulation to characterize as accurately as possible the dose absorbed by organisms and then assess dose-response relationships. The second stage focused on biological effects (developmental endpoints, Transmission Electron Microscopy (TEM), DNA damages and transcriptomics analysis). After exposure to both concentrations, H activity was higher in eggs than in larvae. Comparison between concentrations showed that H 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 showed that CRs of eggs are different, as are those of the larvae. Furthermore, 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 histological differences were observed in the postlarval stage. Furthermore, the expression of some genes related to the transcriptomics and 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 H on zebrafish embryo-larval and, by extent, to the environment.

524 Poster spotlight: TH035, TH036, TH051, TH053

Determining population relevance of ecotoxicological effects

525 The 'so what' question: when is an effect actually an effect? A.C. Brooks, A. Lawrence, Cambridge Environmental Assessments / Regulatory Ecotoxicology

Wildlife risk assessments for plant protection products (PPPs), assessed under Regulation (EC) 1107/2009, are based on data from acute and chronic toxicity tests, and 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 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 Y 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.

526 Population- Relevant Endpoints in Ecotoxicological Hazard and Risk Assessment Endocrine-active Substances (EAS) S. Marty, Dow Chemical Company; A. Blankinship, U.S. Environmental Protection Agency; J. Chambers, Mississippi State University; L.A. Constantine, Pfi brox, Inc.; R.T. Holcomb, Jr., Leibniz-Institute of Freshwater Ecology & Inland Fisheries; A. Kumar, CSIRO / Center for Environmental Contaminants Research; L.L. Lagadic, Bayer AG, Crop Science Division / Environmental Safety; J. Meador, NOAA Fisheries / Ecotoxicology and Environmental Fish Health Program; D. Pickford, Syngenta / Environmental Safety; T. Schwarz, Cef as / Environmental and Animal Health; T.A. Verslycke, Gradient

For ecological risk assessment, endocrine disruptors 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, especially for well-documented historical cases (e.g., bird population declines and DDT; dogfish population declines and TBT). The presentation will be based upon case studies of endocrine-active substances (EAS) (trifluralin, ethinyl estradiol, its metabolite perphenazine, 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 Pelton Workshop™: Environmental Hazard and Risk Assessment Approaches for Endocrine-Active Chemicals. Trifluralin provides a well-documented case-study where population-relevance of endocrine disruption is well established for mollys. 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 evolution 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.

527 Secondary Sex Characteristics - How can changes be interpreted with respect to ED mediated adverse effects? J. Arning, German Environment Agency; E. Fetter, Federal Environment Agency Germany; S. Germer, German Environment Agency; F. Kuckner, Umweltbundesamt / Federal Agency of Environment / IV2.3 Chemicals; S. Frauke, Federal Environment Agency Germany

To demonstrate a biologically plausible link between an endocrine mode of action and 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 characteristics as endpoints. Since they are easier accessible, they are frequently reported in screening tests and non-guideline academic studies. Furthermore, they are often focussed on equating fish and Furan 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 mere adverse/indicative than others? Which weight of evidence must be given to accept changes in secondary sex characteristics as an adverse effect and at which level is it eligible to give secondary sex endpoints more weight. From an analytical perspective, secondary sex endpoints are linked to some difficulties that need to be discussed before coming to regulatory sound conclusions.

528 Modelling breeding success in birds potentially exposed to treated seed A. Lawrence, Cambridge Environmental Assessments / Regulatory Toxicology; 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 breeding season and make a preliminary judgment as to whether a treatment-related effect is likely (EFSF, 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 linnet and skylarks. In all models, this is largely reflected in species vulnerability to TERs. Despite this, we found that populations of skylarks 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 PQR of < 1% reduction compared to a control scenario, or less (including zero reduction).

529 Methods for quantitative weight of evidence assessment of higher tier studies on the toxicity and risks of neonicotinoid insecticides in honeybees K.R. Solomom, University of Guelph / School of Environmental Sciences; G.L. Stephenson, Aquaterra Environmental Consulting Inc. / Environmental Services 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 compare the quality of the available studies from the literature and unpublished reports conducted by the register 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 deviations, this methodology provided graphs and tables illustrating 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

530 Modelling exposure to ionizing substances with spatial and temporal resolved models: A case study for multiple pharmaceuticals in the Baltic area C. Lindin, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; J. van Gils, DELTARES; I.T. Cousins, Stockholm University / Department of Environmental Science and Analytical Chemistry ACES; R. Kühne, Helmholtz centre for environmental research - UFZ / Department of Ecological Chemistry; S. Kutsarova, University Prof. Assen Zlatarov / Laboratory of Mathematical Chemistry; O. Mekenyian, University of Zlatarov / Laboratory of Mathematical Chemistry Water resources contamination by pharmaceuticals is widespread with more than 60 pharmaceuticals detected in surface waters of 7 EU 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 toxic 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 (6x10^3 ton/y) and Ivermectin (2x10^3 ton/y) have discharge loads with higher amounts reaching the Baltic Sea in 2011. 35 of the studied substances had more than 1 ton/y of predicted flux to the sea. Exposure potential given by the ratio of the amount of the drug exported to the sea/amount emitted to the environment was higher than 50% for 7 drugs (Piperacillin, Lorazepam, Metformin, Furosemide and Cetirizine), implying a good potency for these substances. A comprehensive ecotoxicological risk assessment has been conducted and results indicate high risk for Carbazole and 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.

531 European environmental scenarios of chemical bioavailability in freshwater systems

M. Morselli, University of Insbesia / Department of Science and High Technology; G. Morabito, Institute for Ecosystem Study Italian National Research Council; P. van den Brink, Alterra and Wageningen University; F. De Laender, Université de Namur ASBL / Research Unit in Environmental and Evolutionary Ecology; A. Di Guardo, University of Insbesia / Department of Science and High Technology

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 effects. 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 scenarios were performed for a panel of tributary chemicals with (8 PCBs) as well as for the investigated 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 integrating phytoplankton dynamics in both modelling approaches and scenarios for the evaluation of exposure concentrations in aquatic environments.

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532 Evaluation of a global multiscale multimedia fate model framework applied to home and personal care products in Asia

C. Wannaz, University of Michigan; A. Franco, J. Kilgallon, Unilever / Safety and Environmental Assurance Centre SEAC; J. Hodges, Unilever; D. Jolliet, University of Michigan

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 distribution of the substance (PhAT) and the spatial multiscale multimedia fate model Pangea. We used this framework to study emissions associated with HPC products in 17 countries in Asia and Oceania. Spatial distributions of environmental concentrations for 16 relevant substances were obtained using the LCA consensus model USEtox™ (non-spatial), and in a second step with 1600 georeferenced monitoring data for freshwater and sediments collected from the literature. We followed a good practice model definition and testing of the model definition. 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/simplbox. This means that now the nested spatial scale 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 with respect to SimpleBox and other models, which is predicting steady state exposure concentrations for nanomaterials. This is done using a probabilistic approach for calculating PECs for three case studies on TiO₂, Ag and C₆₀ 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.

534 Can release rates be estimated well enough to allow ‘inverse posteriorization’ of REACH substances?

D. van de Meent, RIVM / DMG; J. Meesters, National Institute for Public Health and the Environment RIVM / VSP; E. Tisitsiou, National Institute for Public Health and the Environment RIVM / DMG; D. van de Meent, RIVM / Environmental Science

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 approaches, 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. The model definition has now been incorporated in the original SimpleBox tool and made available as a beta release on rivm.nl/simplbox. This means that now the nested spatial scale 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 with respect to SimpleBox and other models, which is predicting steady state exposure concentrations for nanomaterials. This is done using a probabilistic approach for calculating PECs for three case studies on TiO₂, Ag and C₆₀ 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.

533 Simplebox approach to exposure modeling of nanomaterials - the next step!

J. Truong, University of Toronto / School of Chemical Engineering and Applied Chemistry; C. Yang, University of Toronto / Environmental Monitoring and Reporting Branch; J. Truong, University of Toronto / Chemical Engineering and Applied Chemistry; C. Yang, University of Toronto / Environmental Monitoring and Reporting Branch; J. Truong, University of Toronto / Chemical Engineering and Applied Chemistry

The SimpleBox approach to exposure modeling of nanomaterials is still well adapted to the modeling of the fate of traditional pollutants, but it does not have a high potential to be used as a fast screening tool for various applications. The SimpleBox4.0-nano approach contains the development of a new model, SimpleBox4.0-nano, which is able to calculate mixture toxic pressures in the aquatic environment, comparing the chemical would impair the structure and function of natural ecosystems by resolving 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/simplbox. This means that now the nested spatial scale 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 with respect to SimpleBox and other models, which is predicting steady state exposure concentrations for nanomaterials. This is done using a probabilistic approach for calculating PECs for three case studies on TiO₂, Ag and C₆₀ 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.

535 STREAM-EU: A spatially and temporally resolved exposure assessment approach for pharmaceuticals in aquatic environments

S. van der Elst, Université de Namur ASBL / Research Unit in Environmental and Evolutionary Ecology; G. Morabito, Institute for Ecosystem Study Italian National Research Council; P. van den Brink, Alterra and Wageningen University; F. De Laender, Université de Namur ASBL / Research Unit in Environmental and Evolutionary Ecology; A. Di Guardo, University of Insbesia / Department of Science and High Technology

The STREAM-EU project aims to develop a new exposure assessment approach for pharmaceuticals in aquatic environments. The approach is based on a comprehensive exposure assessment model able to simulate the fate and effects of pharmaceuticals in aquatic ecosystems. The model is designed to be applied at a low resolution, large scale (e.g. global) or with detailed processes and data at high resolution but limited scale (e.g. catchments). The approach includes the following steps: (1) identification and prioritization of pharmaceuticals; (2) development of a model for the fate and effects of pharmaceuticals in aquatic environments; (3) development of a model for the fate and effects of pharmaceuticals in terrestrial environments; (4) integration of the models at the regional scale; (5) validation of the model using field data; (6) application of the model to real-world scenarios. The approach is intended to provide a comprehensive understanding of the fate and effects of pharmaceuticals in aquatic environments, thereby informing decision-making processes related to the management of pharmaceuticals in the environment.
exceedence 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) approach: the ChimERA project (S. Buchinger, Federal Institute of Hydrology / Department G Biochemistry Ecotoxicology; A.M. Bell, Federal Institute of Hydrology / Biochemistry and Ecotoxicology; m. schlesener, T. Ternes, G. Reiferscheid, Federal Institute of Hydrology)
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 correlation. Coefficients for water waste and surface water samples were > 0.9 indicating a satisfying agreement 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
S. L. Kober, TUAS Wildau / Molecular Biotechnology and Functional Genomics; H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis; M. Frohne, TUAS Wildau / Molecular Biotechnology and Functional Genomics
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 contain environmental and health hazards. The most common approach is a combination of HPLC, LC/MS and GC/MS. Validation is needed for tools designed to measure the health impacts of nitroaromatics and to predict health risks for humans and the environment. The statistics, 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 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 also not to suppress the signals of target ions. Previously, 1,5-diaminonaphthalene was shown good properties for the negative ionization of different explosives. Thus, we successfully applied DAN as a matrix for the detection of TNT, 2,4-Dinitrotoluene (DNT), 2,6-Dinitrotoluene (DNT), 2,3-Dinitrotoluene (DNT), 2,4-Dinitrotoluene (DNT) as well as 2,3,5-trinitrobenzene (TNB) and 2,4-Dinitrotoluene (DNB). Without a further separation technique it is not possible to distinguish isomers, but the total amount of ADTs 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 the 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.

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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 its great technical 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 untouched 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+ 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 Ae secondary beam for imaging 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
M.L. Diamond, University of Toronto / Department of Earth Sciences; L.M. Jantunen, Environment Canada / Centre for Atmospheric Research Experiments; P.A. Helm, Ontario Ministry of the Environment & Climate Change / Environmental Monitoring and Reporting Branch; J. Truong, University of Toronto / Chemical Engineering and Applied Chemistry; C. Yang, University of Toronto / Department Earth Science; T.F. Rodgers, University of Toronto / Chemical Engineering and Applied Chemistry; S. Harris, Cancer Care Ontario / Population Health and Prevention, Prevention and Cancer Control
Tris(chloropropyl) phosphate or TCP 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 ΣOPES measured, which were nearly 400 times higher than ΣPBD 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). TCP 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 TCP was estimated to be advected downstream whereas total losses due to degradation was 22% of inputs. Due to TCP’s high solubility, 8% of total emissions was estimated to enter surface waters (streams) via accumulation and wash-off of contaminants in impervious surfaces, followed by wash-off from soils. This input resulted in water concentrations comparable to those reported here. TCP is ubiquitous indoors and outdoors. The relatively high vapour pressure of TCP 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 air is a major environmental sink, as well as a source of 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
The Great Lakes Environment and Climate Change Canada / Air Quality Processes Research Section; L.M. Jantunen, Environment Canada / Centre for Atmospheric
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, polynuorinated diphenyl ethers (PBDEs) and other halogenated flame retardants (HFRs) were monitored in samples collected using high volume air samples (hivols) at 3 land-based stations. Four additional land-based stations using XAD-based passive air samples (PASs) were initiated in 2014. Neutral-per- and polyfluoroalkyl substances (PFASs), organophosphate esters (OPEs) and HFRs were detected in the XAD-PAS samples at levels much higher than the HFRs. OPEs in Great Lakes air were very high compared to open lake samples. OPEs were detectable at all 4 PAS sites. Also, 4 methyl perfluorooctane sulfonamidoethanol (MeFOSE) and methyl pentabromotoluene (PBT). 6:2-, 8:2- and 10:2-fluorotelemers (FTOHs), methyl perfluorooctane sulfonamidoethanol (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 trichloroethylene (TCE) and diphenyl ether (DPE) were only detected 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 eastern 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

540 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. Among 150 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 analysed 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 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 (EWAAS) 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.

541 Indoor Sources of and Human Exposure to Brominated Flame Retardants (BFRs) and Pthalate Esters (PAEs)

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Indoor concentrations of, and exposures to brominated flame retardants (BFRs) and phthalate plasticizers (PAEs) in the Canadian Great Lakes Basin (GLB) were measured in households of individuals known to be exposed to household 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 comprehensively estimate indoor concentrations and potential for exposure 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 (Harnertype 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 (NBRs) in indoor air followed by 2,3- and 2,4-DEP and 2,4-DDE. BDE-209 was the most abundant PBDE congener followed by BDE-99 > BDE-28. PAE abundance was DEP > DnBP > DEHP > DiNP. As expected, dust samples showed different patterns than air. NBRs concentrations fell in the order of TBHP > TBBr > DBDPE. Following the significant dominance of BDE-209 were BDE-99 > BDE-47 > BDE-100 > BDE-138. PAEs concentrations in dust were in the order of DiNP > DEHP > DiBr > BzBr. Hand wipes showed similar profiles of PAEs and NBRs 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.

542 SAFR®: Applying a new hazard and exposure assessment approach for responsible fire safety solutions

S. Admon, IMI TAM Institute for R&D Ltd / Product Stewardship; M. Leifer, J. Tenny, T. Weiss-Coen, ICL - IP

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 retardant materials are an essential part of safe modern living. Nevertheless, fire safety should not compromise safety for human health and the environment. The 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.
544 Spatial variation of secondary inorganic PM2.5 exposure: from exposure magnitude to exposure mapping
K. Stylianou, University of Michigan - School of Public Health / Environmental Health Sciences; C. Tessum, J.D. Marshall, University of Washington / Civil & Environmental Engineering; J. Hill, University of Minnesota / Bioproducts and Biosystems Engineering; O. Jolliet, University of Michigan

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 exposed population and the source characteristics. Estimates for secondary fine particulate matter (PM2.5) IFs - spatial variability in travel distances and PM2.5 exposure due to precursors such as NH3, NOx, and SO2 - are not well defined. We estimate spatially-explicit IFs for NH3, NOx, and SO2, emissions in the 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 PM2.5 IF from ground level NH3, NOx, and SO2 emissions. We also determine how far from the source we achieve x% of total PM2.5 mass change as a result of a precursor emission, mass travel distance of x (MTDx) and x% of total PM2.5 IF as a result of precursor emissions, intake travel distance of x (ITDx). Estimates of PM2.5 intake induced by NH3 emissions indicate substantial spatial variability with values ranging between 0.03 and 14.00 ppm. Sources in CA, TX, GA, FL, and in the East North Central and Middle Atlantic regions show the highest NH3 IFs. Overall, the model estimates that the NH3, NOx, and SO2 contributions each have significant impact on the spatial distribution of PM2.5. The work demonstrates the importance of considering the spatial variability in PM2.5 exposure and its impact on human health.

545 An improved approach for the ecological risk assessment of UVCBs at Environment and Climate Change Canada (ECCC)
M.P. Fernandez, M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division; A. Oksonski, Environment and Climate Change Canada

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 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 vary widely, we use a PBT/vPvB assessment and the risk of the UVCB as a combination of 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.

546 Risk assessment and management of UVCBs with the REACH Regulation
P. Ryan, European Chemical Agency ECHA; R. Cesnatis, P. Karamertzian, European Chemicals Agency

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 other substances falling within the scope of REACH Regulation, substances of <strong>U</strong>-known or Variable Composition, Complex reaction products or Biological materials (UVCBs) 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 physicochemical 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 each constituent. Similar to the approach 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. JUCLID 6 and chemicals tools have been developed for this purpose. Similar to PBT/vPvB assessment, 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.

Report on the RIFM/ECFETOC Workshop: Developing a strategy to improve the environmental risk assessment of difficult to test multi-component substances
J. de Knecht, RIVM; D.T. Saldiva, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; R. Cesnatis, European Chemicals Agency; K. Eisenreich, Environmental Protection Agency / OPPTRAD; M. Embry, HESI; M.P. Fernandez, Environment and Climate Change Canada / Ecological

Improving the environmental assessment of complex composition substances and mixtures for Chemicals
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 iUVCB risk assessment is conducted based on parallel hazard, exposure and risk assessments of the constituents. Upon ECHA’s request, representatives of iUVCB intermediates committed to improve the combined toxicity assessment part of their iUVCB 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 addressed 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 outcome and status of ongoing research 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, nano-biocides and co-contaminants: Fate, effects and risk assessment for biota, the environment and human health (II)**

**550 Do microplastics contaminate the atmospheric environment?**

S. Wright, Kings College London; F. Festy, Kings College London / Tissue Engineering & Biophotonics; F. Kelly, Kings College London / MRC-PHE Centre for Environment and Health

Microplastics - microscopic pieces of degraded plastic litter, or purposefully manufactured microbeads - are a pollutant of increasing environmental concern. Recently, plastic microfibres 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 observed 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.

**551 What happens to polyethylene in the ocean?**

T. M. Karlsson, University of Gothenburg; I. Jakubowicz, SP / Chemistry, Materials and Surfaces; M. Hasselov, University of Gothenburg / Department of Marine Sciences

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

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**549 Addressing combined toxicity in the environmental risk assessment of inorganic UVCBs**

K. Oorts, ARCHE; F. Iacino, Areche consulting; V. VEROUGSTRAETE, Euromaticas / EHS; H. Waeterschoot, Euromaticas

There is a need to develop an integrated approach to address combined toxicity when assessing multi-metallic substances such as inorganic UVCBs (iUVCBs). Because

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**548 Unknown or Variable Composition, Complex reaction products or Biological materials and still the same: is this possible?**

E. Vaipouolu, CONCAWE; K. den Haan, CONCAWE / Petroleum Products & Safety; E. Boix, CONCAWE; P.R. Fisk, Saxon House; B. Barnes, S. Summerfield, Peter Fisk Associates Limited

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. UVCB 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 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-MS, 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 outcomes can be related to the different strategies of analysis of the variability of composition 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 groupings can be used to determine the number of clusters that may possess 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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**547 Assessing the Biotic Toxicity of UVCBs in an Environmental Risk Assessment Framework**

J. Lee, E. Vaipouolu, CONCAWE; K. den Haan, CONCAWE / Petroleum Products & Safety; E. Boix, CONCAWE; P.R. Fisk, Saxon House; B. Barnes, S. Summerfield, Peter Fisk Associates Limited

Addressing combined toxicity in the environmental risk assessment of inorganic UVCBs

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**546 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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**545 Microplastics, nano-biocides and co-contaminants: Fate, effects and risk assessment for biota, the environment and human health (II)**

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**SETAC Europe 27th Annual Meeting Abstract Book**
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 placed on different sites to four different levels and added to stainless steel cages hanging in the water. Samples were taken every four weeks and assessed to analyze 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 in biodegradation risk assessment and to develop appropriate methods to predict and monitor the transportation and ultimate fate of plastic material in the ocean.

552 Marine snows: a vector of transport of microplastic to the benthos and its biological implications
A. Porter, University of Exeter / Biodisciplines; A. Watts, University of Exeter; B.P. Lyons, Cefas Weymouth Laboratory; T.S. Galloway, C. Lewis, University of Exeter / Department of Biosciences

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 looked 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 benthic plastic. 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 tablettoppler. 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 snow 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.

553 Desorption of organic wastewater contaminants from microplastic particles in presence of natural dissolved substances
S. Seidensticker, G. Fellenberg, University ofTuebingen / Center for Applied Geosciences; C. Zarfl, University of Tuebingen / Center for Applied Geosciences; P. Gehrwohl, Uni Tuebingen / Center for Applied Geosciences

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 microplastics is largely unknown, e.g. to the effect of further contaminants in 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 acids (HA) as a common type of dissolved organic matter 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 surface and to study HA in depth. After 180 min stirring, MP and HA in duplicates were filtered on 0.45 μm filters. 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, toluene, and 4- and nonylphenol (4-and NP), respectively. The mean log partition coefficients between HA and water were, 3.74 for phenanthrene, 3.92 for toluene, and 4.02 for 4-n-NP. In equilibrium, increasing amounts of HA go along with a decreasing fraction sorbed to MP and an increasing 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 rate of sorption for formaldehyde of HA are present. With increasing amounts of HA, desorption kinetics was more and more governed by interpolymer 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.

554 Do microplastics really act as vectors of organic pollutants to marine zooplankton? Experiments with pyrene and nonylpheno spided 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 toxins to planktonic filter feeders. We have tested this hypothesis using a standard marine toxicity test, the sea-urchin embryoto 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: 1/10 x and 1 x the no-observed-effects level (NOEL) in both 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 planktonic.

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

555 Harmonizing human exposure and toxicity characterization
P. Funte, Technical University of Denmark / Quantitative Sustainability Assessment Division; O. Jollivet, University of Michigan; T.E. McKone, University of California / School of Public Health

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 integrating toxicity dose-response functions. To extend the applications beyond 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 outcomes, 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.

556 A method for application-specific human health risk estimation from chemical exposure in an LCA context
exp. Edsberg, J. Lexén, Christer Lindholm, E. Lundström Belleza, IVL Swedish Engineering & Biophotonics; F. Kelly, Kings College London / MRC-PHE Centre for Environment and Health

SETAC Europe 27th Annual Meeting Abstract Book
Environmental Research Institute: Q. de Hults, BASF Belgium Coordination Center: V. P. Salig, BASF SE / Sustainability Strategy: P. Coirs, E. van Miert, Solvay SA; A. Wathete, Solvay SA / LCA; T. Tiemersma, X. Weinbeck, DSM Engineering Plastics; M. Glöckner, Deutsche Bauchemie; N. Amann, Wacker Chemie; P. Ashford, R. Downes, Anthesis Group; D. Koelsch, Bayer Technology Services GmbH; N. von der Assen, Bayer AG; C. Karafyllidis, Covestro Deutschland AG

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 fulfill this need based on a systematic and practically 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 and 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 expressed 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). This relates the amount of the contaminant reaching 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 smelth structure allows for comparisons 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.

557 Learning from LCA-based methods: should chemicals in food packaging be a priority focus to protect human health? A. Emsboff, Quantis / Quantitative Sustainability Assessment; K. Stylianou, University of Bordeaux / ISM CyVi; O. Jolliet, EPFL; Q. de Hults, BASF Belgium

In this paper, the focus is on consumer exposure during use phase is essential for both LCA and ACC, the demonstration the feasibility of combining chemical specific Life Cycle Inventory requirements, challenges and consequences

558 Modeling indoor occupational air emissions of nanomaterials for life-cycle assessment M. Tsang, US EPA / ASPH Fellow / ISM CyVi; D. Li, K. Garner, University of California, Santa Barbara / Bren School of Environmental Science and Management; A. Kessler, University of California, Santa Barbara / Bren School of Environmental Science and Management; S. Sub, University of California, Santa Barbara / Bren School of Environmental Science and Management; G. Sonnemann, University of Bordeaux / ISM CyVi

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 the potential environmental and human health risks caused by chemicals in packaging, and vice versa that the retained-intake fraction was inversely related to the emission magnitudes, which is counter-intuitive to conventional thinking in LCA.

559 Towards a Life Cycle Based Chemical Alternative Assessment (LCAA) O. Jollivet, University of Michigan; L. Huang, University of Michigan / Dept of Environmental Health Sciences; M. Overcash, Wichita State University / Industrial, systems, and Manufacturing Engineering Department; P. Fantke, Technical University of Denmark / Quantitative Sustainability Assessment Division; A. Aaurait, Quantis; A. Kounina, Quantis / EPFL; O. Jolliet, University of Michigan

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 sugar sweetened beverages (SSB) packaged in high-impact polystyrene (HIPS) due to various relevant health impacts. Specifically, we aim to assess 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 capita” in the United States.” Method developments focus on the 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 overall minimum risks 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 over minimum risk are high priority for mitigating HHD, as well as associated chemical, packaging, food, scenario, and HHD was extrapolated following 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, phagocytosing 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.

Environmental risk assessment of biocides: regulatory requirements, challenges and consequences

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560 Introductory talk: Working groups involved in the environmental assessment of biocides and latest developments of guidance and guidance-related documents

H. Schimmelknopfer, European Chemicals Agency; ECHA / DI - Biocides; S. Gerasimou for interpretation)

Therefore, the German environmental agency ECHA-European Chemicals Agency / Biocides; J. Lautin, ECHA-European Chemicals Agency; A. Lipkova, European Chemical Agency ECHA; e. noegheco, ECHA; J. Weber, European Chemical Agency ECHA

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 the ECHA’s BPC guideline. 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 been 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) and not all validation of the 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.

561 Comparative environmental risk assessment of biocides - a chance to get better products

L. Rankes, University of Bremen; A. Coors, ECT Oekotoxikologie GmbH; P. Vollmar, ECT Oekotoxikologie GmbH; C. Riedhammer, German Environment Agency Umweltbundesamt

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 (CIS) 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. The 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 discussion about its definition and not all validation of the emission scenario document (ESD) 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.

562 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 to be 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. MANPEC version 3.1 was used to calculate Predicted Environmental Concentrations (PEC) of dichloroanid and ragreral 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 MANPEC representative for all Danish marinas appears to be a challenge as the marinas are not identical and not all validation of the emission scenario document (ESD) 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.

563 Studies to support the national environmental risk assessment on antifouling products for leisure boats

M. Fes MAGIC, Umweltbundesamt; B.T. Watermann, D. Daune, C. Fueter, A. Thomsen, LimnoMar

Within the authorisation of biocidal antifouling products for leisure boats (EU Biocide Regulation 528/2012) national specifics may be considered by the member states in their assessment of environmental risks on antifouling products (AP). 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 Environmental 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 breakdown 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 AP, biocidal substances and their breakdown products. It was recognized that national biocidal 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) and not all validation of the emission scenario document (ESD) 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.
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 deposited 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 performance 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.
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 earthworm Lumbricus rubellus has successfully adapted to this heavily polluted environment. Exploring this and other extremeophiles 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 as 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 observed for the previously exposed specimens compared to control specimens. 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 pharmacological 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 imagines 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 imagines, 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 edecysone signalling potentiation and stress response mechanism over-representation, explaining the phenotypic modifications observed in the progeny of exposed parents. Overall, our study illustrates the (RNA) proof of concept in mosquito to identify the underlying molecular mechanisms. In summary, this study provides new evidence for carcinogenic potentiality of ibuprofen biomarkers and the potential role of integrated OMICS epigenetics that process.

MO003
Integrated transcriptomics-epigenomics modulations in Graphene oxide nanomaterials’ mediated transformed cells
N. Chattjee, University of Seoul / Environmental Engineering; C.P. Roca, University of Aarhus / Department of Bioscience; J. Choi, University of Seoul / School of Environmental Engineering
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 (mRNA and DNA methylation) profiling to exposed to control and polluted soils. These investigations, for the first time, 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
R. Duca, KU Leuven / Center of Environment and Health; N. Grova, Luxembourg Institute of Health / Human Biomonitoring Research Unit; M. Ghosh, KU Leuven / Environment and Health; B.M. Appenzeller, Luxembourg Institute of Health / Human Biomonitoring Research Unit; J. Vanrobaeck, KU Leuven / Environment and Health; L. Goddiers, KU Leuven / Environment and Health
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 chronic 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 hometic response may give critical insights into the PAH epigenetic carcinogenicity.

MO005
Copper induces expression and methylation changes of homoeotic genes in Crassostrea gigas embryos
M. Assenasiadi, M. Lebre, L. Rouxel, F. Akcha, IFREMER / Laboratoire détoxicoécologie; G. Rivièrè, Université Caen-Basse Normandie / UMR BOREA
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 four nominal concentrations of copper (0.1, 1, 10 and 20 µg/L). Embryotoxicity was monitored at 24 h post fertilization (hpf) and genotoxicity at 7 hpf, respectively at D-larvae and gastrula stage. In parallel the relative expression of 16 genes coding for homoeotic genes and involved in biomeralization 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, while significant genotoxic effects at a lower concentration of 1 µg/L. All the genes presented a stage dependent expression pattern, and the expression levels of four homoeotic genes (Notochord, HOX1A1, HOX2, Engrailed 2) were modified following copper exposure. Global DNA methylation (5-Methylcytidine and 5-Methylcytidylic acid) 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, HOX1A1, HOX2, Engrailed 2 genes in control samples but not in copper exposed ones. Our study suggests that the embryotoxic effects of copper on Pacific oysters could be partly mediated through homoeotic gene expression impairment through changes in DNA methylation.
**MO006**


Increasing evidence demonstrates that environmental insults occurring during the early development may have long-life 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, for the first time, to evaluate the Endocrine Disruption Chemicals (EDCs) on zebrafish development by combining metabolomic, transcriptomic and epigenetic 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 transcriptional 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 dpf, and analyzed changes in gene expression patterns by RNA-seq (RNA-seq) and in the metabolome by untargeted LC-MS. We found alteration of the retinoid and thyroid pathways and have selected a set of regions of brain aromatase (cyp19a1b) and aldolase dehydrogenase 1 family member A2 (aldha1a2) 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. 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 ecotoxicoeugenetics 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. S. Prud'homme, Edysan FRE 5408 CNRS - UPFV / Laboratoire dEcologie Alpine LECA, a chaumet, Istria/ UK MALY Laboratoire Ecotoxicologie; E. Cassar, J. David, S. Reynaud, Université Grenoble Alpes UMR CNRS / Laboratoire dEcologie Alpine LECA

Human activities lead to chronic contamination of surface waters by micropollutants. Although measured concentrations of such pollutants are often such that detection using conventional tests, 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 benz[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 progeny of individuals chronically exposed to 1 µg/L ibuprofen or 0.6 µg/L benz[a]pyrene. The propensity of individuals exposed to ibuprofen had an accelerated development while the progeny of individuals exposed to benz[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 small increase of A. aegypti asymptotic population growth rate. Multigenerational exposures for two generations revealed an evolution of population response to ibuprofen and benz[a]pyrene across generations, leading to a loss of previously identified transgenerational effects and to the emergence of a tolerance to the biocides Bacillus thuringiensis israelensis (Bti). This study shed light on the short and long term impacts of environmental contamination on Aedes aegypti life-history traits and insecticide tolerance, raising unprecedented insights 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. S. Choi, University of Seoul / School of Environmental Engineering; N. Chatterjee, University of Seoul / Environmental Engineering; Y. Gwak, J. Choi, University of Seoul / School of Environmental Engineering

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 piperonyl 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 progeny 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 etc.) 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 persistence and 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 magna, 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. J. Yang, University of Seoul / School of Environmental Engineering; N. Chatterjee, University of Seoul / Environmental Engineering; Y. Kim, University of Seoul / School of Environmental Engineering; J. Roh, Division of Environmental Science and Ecological Engineering / Division of Environmental Science and Ecological Engineering; J. Kwon, Korea University / Division of Environmental Science and Ecological Engineering / Environmental Health Center; J. Choi, University of Seoul / School of Environmental Engineering

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 nematode, Caenorhabditis elegans: An epigenetic approach with various exposure scenarios. We have found alteration of the retinol and steroid pathways 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: Crude oil, Caenorhabditis elegans, Reproduction, Differential exposure, Epigenetics, Histone methyltransferase

**Advances on the assessment of environmental pollutants to amphipods 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. S.D. Melvin, Griffith University / Australian Rivers Institute; L.J. Habener, Griffith University / Griffith School of Environment; F.D. Leusch, Griffith University - Smart Water Research Centre / Griffith School of Environment; A.R. Carroll, Griffith University / Griffith School of Environment

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 antibiotics, anti-inflammatory 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 (Lithobates peronii) tadpoles to control, solvent control (methanol), and a mixture of the drugs metformin, atorvastatin, and bezafibrate at 0.5, 5, and 50 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?**

B. Rabelo Costa, Department of Biology & CESAM - University of Aveiro / Biology; I. Lopes, University of Aveiro / Department of Biology & CESAM; 3810-193 Aveiro

Gold nanoparticles are commonly used in medical therapies 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 determine 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 10^3 cells/mL of the microbialae *Raphidocelis subcapitatus* 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 (EC50), 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, 0.01mg/mL: 4.28±0.05 mm and highest tested concentration, 0.01mg/mL: 3.64±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±1.17 mm) comparatively to those exposed to 0.007 (11.56±1.10 mm) and 0.01(10.90±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**

L. Gonçalves, University of Aveiro; F. Fonseca, University of Aveiro / Department of Biology / Centre for Environmental and Marine Studies CESAM; S.M. Marques, University of Aveiro

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 showed that these organisms are very susceptible to water and air contaminants, there are many unknowns about how these factors can interact and affect amphibian populations. In this study we analyzed 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 ecothermic 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 or metribuzine. The environment of these sites, 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, we performed embryonic and larval exposure experiments with ibuprofen and benzo[a]pyrene. The concentration ranges used were: 0.007 (11.15±0.10 mm) and 0.01(10.80±0.08 mm) mg/ml of Au-NR. Finally, 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±1.17 mm) comparatively to those exposed to 0.007 (11.56±1.10 mm) and 0.01(10.90±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.

**MO013 Tolerance of amphibians and its skin symbiotic bacteria to increased salinity**

A.C. Gabriel, University of Aveiro / Biology; S. Costa, Aveiro University CESAM / Department of Biology; I. Lopes, University of Aveiro / Department of Biology & CESAM; 3810-193 Aveiro

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 EC50 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 (EC20±SD) 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 (LC20±SD) was 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 EC10 for NaCl, being the EC50 (95% confidence limits), before and after such exposure, of 20.18 (25.5-21.9) 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 metabolism 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, evolve new characteristics, requiring other metabolic mechanisms to tolerate salinity stress.

**MO014 Interactions of environmental stressors with the amphibian pathogen *Saprolegnia australis***

S. Costa, Aveiro University CESAM / Department of Biology; C. Félix, Aveiro University CESAM / Department of Biology; J. Costa, Aveiro University CESAM / Department of Chemistry; T. Rocha-Santos, University of Aveiro CESAM / Department of Chemistry; A. Estevos, Aveiro University CESAM / Biology department; A. Alves, CESAM University of Aveiro / Department of Biology; I. Lopes, University of Aveiro / Department of Biology & CESAM; 3810-193 Aveiro

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, ecologicaltoxicological assays were carried out by exposing the pathogen to: i) varying NaCl concentrations (from 4.9 mM to 5.49 mM); ii) seawater dilutions (SW), equivalent to each tested NaCl solution; iii) acid mine drainage (AMD) dilutions (from 0% to 100%) and iv) varying pH values, with no changes to NaCl concentrations. The results showed that the AMD pH value contributed to increased biomass production, extracellular enzymatic activity and mycelium chemical composition
Methyl ethyl ketone peroxide, vinyl chloride, methylhydrazine, hydrogen cyanide, and 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-dinitrophenol, cresol, benzene, methyl ethyl ketone peroxide, vinyl chloride, methylhydroxide, 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 eco-toxicity data of terrestrial ecotypes up to date. We searched and listed the available eco-toxicity 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’.

MO015 Current studies on ecotoxicity of accident preparedness substances in Korea Y. Choe, Konkuk University; Y. An, Konkuk University / Department of Environmental Health Science
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 (inhalation hazard, skin hazard, eye hazard, ingestion hazard, nausea, vomiting, diarrhea, labored breathing, chemical burns, cyanosis, dizziness, disorientation, drowsiness, and unconsciousness). Among the substances, hydrogen peroxide, phosgene, 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-dinitrophenol, cresol, benzene, methyl ethyl ketone peroxide, vinyl chloride, methylhydroxide, 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 eco-toxicity data of terrestrial ecotypes up to date. We searched and listed the available eco-toxicity 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. Aube, 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 M. Van Ginneken, University of Antwerp, Dept. Biology / Biology (SPHERE); R. Blust, University of Antwerp; L. Vervoets, University of Antwerp / Biology. 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 echinoderm Asellus aquaticus (Isopoda). As metal concentrations, we chose the EQS, 10xEQS and 100xEQS, for Cd: 0.15, 1.5 and 15 μg L⁻¹; for Cu: 7, 70 and 700 μg L⁻¹; for Pb: 130 μg L⁻¹ for Cd: 7.2 and 720 μg 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, Symbiodiophyllum spp., and their combined toxicity with global warming. A. Tagliati, Heriot Watt University / Institute of Life and Earth Sciences; S.J. Hennige, The University of Edinburgh / School of Geosciences; T.F. Fernandes, Heriot-Watt University / Institute of Life and Earth Sciences
Titanium dioxide nanoparticles (TiO2) are widely used as UV-filters in sunscreen. The chosen nTiO2 share the same crystal structure (rutile) but have different external coatings (dihemichrome, simethicone and stearic acid). Diverse Symbiodiophyllum phylotypes, known for their different tolerance to environmental change and stress, were exposed to nTiO2 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 nTiO2 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 the coral tissues is considered as the trigger to coral bleaching. Thus the observed enhanced ROS production in the algal cells, derived by the simultaneous exposure to nTiO2 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 A. Varela, University of Leuven; L. Janssen, 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 and 24°C) and, when they reached the final larval stage, exposed to one of two chlopyrifos 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 and reduced size at metamorphosis, pesticide exposure did not affect size. As expected, the effect of chlopyrifos 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 those observed. 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 of the offspring, with effects on 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 A.R. Santillán-Sarmiento, J. Moody, Plymouth University / School of Biological & Marine Sciences (Faculty of Science & Engineering); A. González, A. Moenne, Universidad de Santiago de Chile; F. Moenne, C. Saez, Universidad de Playa Ancha / Laboratorio de Investigación Ambiental Costera (LACER); M.T. Brown, Plymouth University / School of Biological & Marine Sciences (Faculty of Science & Engineering)

MO021 Combined effects of invasive species and insecticide exposure to freshwater benthic invertebrates: a mesocosm approach

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Pesticide contamination and invasive species are two main anthropogenic stressors of concern in freshwater systems. Thus, understanding their combined effects to community structure and vital ecosystem functions presents challenges for an improved ecological risk assessment. This study focuses on community-level data obtained in artificial microcosms to evaluate the 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 activity exposure caused a reduction of 4 and 10 % of shredders and collectors, respectively, with consequent reductions in leaf decomposition. These deleterious effects 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 Chironomus. Therefore, this indirect effect of CAP on Eucalyptusleaf and crayfish was present. The presence of the voracious crayfish decreased detritivore abundances, being collectors and grazers the preferential prey. Interestingly, under CAP exposure invertebrate mortality due to crayfish predation seemed to be reduced, which might be related to CAP induced effects on behavioral activity. In conclusion, this study highlights the influence of leaf litter type on shredder abundance and primary productivity, 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 invertebrate 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.

C. Nielsen, The University of Exeter; C.M. Hird, C. Lewis, University of Exeter / Department of Biosciences

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 many of the common metals such as Cu are toxic to 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 pCO2. This suggests that these worms are responding to OA 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 pCO2 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 Zn2+. 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 ad hoc P supplies where the effects are unclear and not known. 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. magnaeureproduction 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. Therefore, we examined how dispersal of 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 diversity and productivity, while toxic species richness increased under experimental 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 showed 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 from 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 bloom of blue-green algae

Nakdong (ND) and Yeongsan (YS) river, two of 4 major rivers in Korea, are suffered from blue-green algae in hot season. Interestingly, YS river with greater nutrient levels (i.e., higher nitrogen/phosphate 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 high resolution method based on SPE-RLC-HRMS platform. 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 (Acloroh, Diuron, Mefenacet, Oxadiazon, Simazine), 2 Insecticides (Chlorpyrifos, Diazinon). The median concentrations of pesticides in Nakdong/Yeongsan rivers are as follow: Iprobenfos 178/382 μg/L, Tebuconazole 24/58 μg/L, Acloroh 49/71 μg/L, Diuron 22/63 μg/L, Mefenacet 142/296 μg/L, Oxadiazon 75/578 μg/L, Simazine 7/5 μg/L, Chlorpyrifos 178/382 μg/L, Diazinon 5/7 μg/L. The Sum of the median value in YS (1,755 μg/L) river is about 3 times greater than that of ND river (597 μg/L). Although integrated effects of identified pesticide cocktail on algal growth is not well estimated, the significant 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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Spatial and temporal relationships reveal the impact of dams on river ecosystem resilience: The Ebro river (NE Spain) as case study

2 Persistent Organic Pollutants (POPs) are distributed globally through long-range transport 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, abundance and biomass 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 discontinuities on the river ecosystem associated to the presence of dams (b) the relative contribution of synchronization vs. local dynamics processes and (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-RED). The research leading to these results has received funding from the European Communities 7th Framework Programme under Grant Agreement No. 605629-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 novaengliae) feeding in Antarctica have been found to accumulate large amounts of HCB in their adipose tissue. During their long migration they undergo decreases of volatilisation due to a decrease of 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 hydrophobic nature, which led to problems with cell integrity 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
A sampling campaign was undertaken parallel for both river from June to October.

Water Quality Assessment Research Division; J. Jeon, Changwon National University.

The relevance to blooms of blue-green algae (cyanobacteria) and zooplankton community in the Belgian part of the North Sea. Hence, we have available material related to phytoplankton. To partly address this knowledge gap, in the laboratory, we conducted exposure experiments with species representing a gradient from unpolluted to polluted communities. The results of this study were used to estimate the availability of toxicants in 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 results were used to determine the toxicity of the metals.

MO029
Methylmercury in Arctic Seabirds - Seasonal inter-tissue differences
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Mercury (Hg) is a natural element, however, anthropogenic activities have introduced it to the environment, and it has accumulated in different ecosystems. The relevance of methylmercury (MeHg) to human health is significant, as MeHg is bioaccumulated in the food web and can reach high concentrations in organisms at the top of the food web. The focus of this study was to quantify the accumulation of MeHg in local, regional, and global biodiversity. The results of this study were used to estimate the availability of toxicants to the Baltic Sea ecosystem.

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. Environmental gradients are driven by global fractionation processes and by increasing remoteness from POP sources. The occurrence of organochlorines (OCs) and polybrominated diphenyl ether (PBDEs) was analyzed 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 skua colonies, and differed significantly across colonies. Contrary to the expected spatial gradient, the second most remote colony located at the second highest latitude (Bjornoya), had the highest contaminant concentrations. The relative pattern also differed among colonies; while Shetland great skua having a slightly higher proportion of heavy and less volatile 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 skus' migration strategies and diet sources, rather than long-range transport, may explain their contaminant occurrence.

Wildlife ecotoxinsology: cumulative effects from 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 inheritance of pollution impacts 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 secondary stream, were sampled at four different trophic levels: water, sediment, invertebrates and fish. All samples were subjected to an optimized protocol for the detection 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 glutathione S-transferase. Sanger sequencing of primer pairs were developed and have now been evaluated suitable for real time qPCR analysis. First results suggest an overall stronger expression of catalase in comparison to the other four genes of interest, while the difference among sites was less pronounced.

MO032
A little bird once told me: Health effects 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 (NOx) and many other factors. Motor vehicles are the major source of NOx in the USA, therefore, in an effort to improve air quality, environmental regulators such as the US EPA have set increasingly stringent standards for vehicles emissions and fuel quality. Since 1990, in the USA total NOx emissions have nearly halved and hydrocarbons (such as benzene, toluene, ethylbenzene and xylene, 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 NOx may have been featured as the major contributor to negative health effects in the past, the dramatic reduction in NOx concentrations may reveal that the other products of fossil-fuel combustion such as benzene or toluene, are emerging as important contributors. Despite the importance of this question, little is known about the impact of NOx on health at low concentrations. The current investigation was designed in two parts to address this deficit. Part 1 examined the effects of urban air pollution on nesting 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 these investigations will be evaluated against those of a similar study published in 2016, suggesting that BTEX, not NO2, 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 change when assessing 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 too late can be too costly to recovery. Here, we present an example in a fish species if juvenile fish, for which the potential impacts on population process is needed. In the UK, the Predatory Bird Monitoring SETAC Europe 27th Annual Meeting Abstract Book 127
MOO34 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 (2PCB) and dichlorodiphenyltrichloroethane (DDT, 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 2PCB and p,p'-DDT concentrations than adults, which in contrast presented higher levels of p,p'-DDE than nestlings. Levels of 2PCB significantly increased with a “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 OC exposure in wildlife. Levels of p,p'-DDE significantly increased with the proportion of wetlands within the breeding territory. No association was assessed with the proportion of electric transformers around active nests, which we propose as a useful tool for assessing electric transformers around active nests, which we propose as a useful tool for assessing environment condition and the year when they were sampled or captured revealed an important inter-annual variability. Decreased levels of DDE and increased levels of PCB were also detected in older birds (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(loids) 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).

MOO36 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 systems. 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 avian 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.

MOO37 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 population is bullet fragmentation from big game hunting and ingestion of lead residues by birds of prey. Stable isotope analyses have allowed several authors to identify lead ammunition as the major source of lead exposure for these birds (Pain et al., 2007; Berry et al., 2015, Madrý 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

MOO38 Immunological 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) is an endemic species of the tropical temperate Atlantic Ocean, with a widespread distribution. Although 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. Therefore, 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, chlorodanes, 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 containing with important data that could be considered in future local conservation programs.

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

MO039 Uptake of emerging contaminants under hydroponic condition E.M. Beltrán, INIA - National Institute for Agricultural and Food Research and Technology / Environmental; M. Gonzalez-Doncel, National Institute for Agricultural and Food Research and Technology of the Environment; M.A. Porcel, INIA National Institute for Agricultural and Food Research and Technology / Department of Environment Laboratory for Ecotoxicology; I. Atiénzar, INIA National Institute for Agricultural and Food Research and Technology / Department of Environment; G. Carbonell, C. Fernández, INIA National Institute for Agricultural and Food Research and Technology / Department of Environment

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 apical meristem) were transferred onto a nutrient solution 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 Competitiveness projects CTM2013-44896-R and CTM2014-52388-R

MO040 Assessment of sources, occurrence and fate of the fungicide carbendazim in water S. Merol, S. Benzing, C. Gleiser, S. Leje, J.E. Yanez Heras, S. Tieder, University of Tuebingen / Environmental Analytical Chemistry, Center for Applied Geosciences; C. Zwiener, Environmental Analytical Chemistry, Center for Applied Geoscience, University of Tuebingen / Geosciences

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 and 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 carbendazim in wastewater effluents from Germany during dry weather periods. Paper and textile samples 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 (towels 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 in the housing estate 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 were identified as the acid (97%-1,8-dihydroxy carbendazim), the aldehyde (17d-estradiol), the hydroxylated (17β-estradiol) and the corresponding glucuronides. 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 suitable approach to investigate the occurrence and fate of major transformation products of carbendazim.

MO041 Biodegradation of 3 pharmaceutical compounds in activated sludge C. Fernández-López, UCAM Catholic University of Murcia / Water and Environmental Research Group; B.A. Poursat, University of Amsterdam/IBED Institute / Institut für biodiversität und ecosystem dynamics; R. Helmus, J. Parsons, University of Amsterdam / IBED

Different tests were carried out to determine the biodegradability of three pharmaceutical compounds (PCs), one anti-epileptic drug (carbamazepine), an anti-inflammatory compound (naproxen) and the steroid and sex hormone (17β-estradiol). PCs, the mineralization tests were based on OECD guideline 310 (Ready Biodegradability - CO2 in sealed vessels (Headspace Test) 2006) and the testing period was 28 days, with CO2 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.


Psychotropic 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, metabolism 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 were detected. 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, with higher levels of venlafaxine and citalopram. For carbamazepine, no bioaccumulation was found. For the two other TPs, no significant difference was found between TPs with and without the treatments. For the bioaccumulation, it was found that warming conditions increased the bioaccumulation of the three compounds, while acidification decreased the bioaccumulation of venlafaxine and citalopram. For carbamazepine, warming conditions decreased the bioaccumulation, while acidification increased the bioaccumulation of carbamazepine. For the depuration, warming conditions decreased the depuration of carbamazepine and citalopram, while acidification increased the depuration of both compounds.
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, depression, convulsion, insomnia or panic disorder, being Diazepam one of most prescribed. In the same way as other pharmaceuticals, these compounds can enter wastewater 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 occur generating 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 benzo diazepines has not been performed so far. The aim of this work was to study the reaction between chlorine and diazepam and three related compounds (nordiazepam, 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 (QTFQ). For those benzo diazepines which showed a significant degradation, i.e. diazepam, oxazepam and nordiazepam, parameters affecting to the degradation reaction (pH, chloride and bromide content) were studied in detail using an experimental design. Moreover, 9 TPs were tentatively identified. The transformation of the studied benzo diazepines led mainly to the formation of benzophenone or quinazoline derivatives. Finally, the evaluation of their ecotoxicity was carried out using quantitative structure-activity relationship (QSAR) software tools: Ecological Structure 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. Kosiej et al. Water Res 6 (2011) 355–368, [2] C. Postigo I Hazard Mater 279 (2014) 461–475.

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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 genotoxic than the initial chemicals. Evaluation of this risk is especially important for compounds that may persist in the environment without effective degradation, for example, due to the pressure of organisms 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 fluorquinolones, tetracycline and chloramphozine 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 Zagdzaj (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 enable rapid discovery of binders against a wide range of targets. Affimers1 are a recent example of AMs; inspired by the discovery of natural Immunoglobulin-like molecules, the aim of this technology is to exploit the potential of Affimers as suitable bio-receptors for the detection of environmental contaminants, such as methylene blue and dichlofenac. 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 screening strategies based on regulatory databases
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Discharges from sewage treatment plants (WWTP) and also small-scale on-site facilities (OSSFs). OSSFs are in some cases more toxics than precursor drugs. It’s worth mentioning that the study of the reaction of chlorine with benzodiazepines has not been continuously measured during the rain events in order to put into correlations to the aquatic environment. It includes conventional wastewater treatment plants (WWTP) and also small-scale on-site facilities (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 perfluoralkyl substances (PFAs), 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 affected by the presence of OSSFs and WWTPs. In all cases, both the monitored 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 consumer 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. Previous studies reported the transport of pharmaceuticals and personal care products from a populated area to the oceanic environment via regional runoff. For chemical analysis, the effluent samples and the receiving seawater around the

SETAC Europe 27th Annual Meeting Abstract Book
MO048
Emiison of synthetic musk fragrances in surface waters during rain events in urban environments.


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 water and waste waters. The method, validated taking carefully into account the problem of blank contamination, allows to determine 9 compounds, including nitro-, polycyclic- and 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 (2000/60/EC) which recommends to determine some EACs in urban waters. In Belgium, the Zenne River is a river with a high sewage inflow. In this study, a list of EACs was proposed on the basis of EDB 2012 and HSE 2014 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 balance of the pollutant load and track the (highly dynamic) interaction along the river to assess sources and sinks otherwise hidden by classical discursive approaches. These pollutants were based on the total estriol 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 (2015). 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 in flow). Creatinine, Galaxolide lactone (gal), Fetal Alcohol Syndrome (FAS), and E1-eq. were 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 and hospital effluent ranged from 17.6 to 359ng E2-eq./L. In total, 9.97%-15% of the BEQ-estriol activity in 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 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 particulate material, 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, merformin, 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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Technology; H. Horn, Karlsruhe Institute of Technology KIT / Engler Bunte Institut Chair of Water Chemistry and Water Technology
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 concentrations. 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/m²) 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

K. Lee, Plymouth University / Biogeochemistry Research Group; M. Fitzsimons, Plymouth University; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; A. Tappin, Plymouth University; S. Comber, Plymouth University / Environmental Sciences.

Water reuse 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 wastewater application. As such, we have developed analytical methods to detect and quantify APIs in these conditions. We have also recently developed an area that requires increased attention. Terrestrial risk assessments are also not conducted for APIs which have an organic carbon partitioning coefficient (K ow) > 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 propanolol 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 soil on API fate and transport was studied at pH conditions in line with conditions in wastewater and at pH conditions 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.

**MO054**

In situ measurement of nanoparticles (P)

C. Stephan, PerkinElmer, Inc.; J. Wilkinson, Kingston University London / Natural and Built Environments; M. Hadjiv, Universite de Montreal

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 effects1 that should, ideally, be performed by in situ analysis and give physicochemical characterization. This is mostly not achievable due to technical limitations for environmental matrices since nanoparticle concentrations are typically very low2. Historically, particle size has been measured by Dynamic Light Scattering (DLS) and Transmission Electron Microscopy (TEM), while dissolved content has been measured by ultratillation followed by indirectly 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 study is to investigate the use 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

G. AMARIEL, University of Alcalá; J. Santiago-Morales, University of Alcalá / Chemistry I; K. Boltes, University of Alcalá / Chemical Engineering; P. Letón, University of Alcalá; A. Rodríguez Fernández-Alba, University of Almeria / Department of Chemistry; R. Rosal, University of Alcalá. The nanotechnology revolution has a great potential to enhance a wide variety of products and services. In water treatment sector, particularly 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 product1. However, the promise can be offset by the concern of poor polymer biodegradability and the need for material 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)3. The modern nano-chemistry has demonstrated that dendrimers can be an effective solution, by combining their unique structural characteristics with a range of physicochemical functionalities1. In this work, we prepared PAMAMG3-NH2, 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., Pluta D.I., Hutchinson 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., Amarieli 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. Acknowledgement - This work has been financed by the FPT-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.

**MO056**

Photocatalytic decomposition of diclofenac sodium in aqueous solution by nanoparticle mixture ZnO/TiO2

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Diclofenac sodium, 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-([5-dichlorophenyl]amino)phenylacetic acid, DCF) is commonly used NSAID, resisted to biodegradation and added 15% of DCF is excreted during its usage. The heterogeneous photocatalysis based on interaction of UV irradiation and semiconductor was studied in decomposition of diclofenac by nanoparticle mixture ZnO/TiO2, ZnO/TiO2; 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 experiments were conducted in bactericide and high turbidity 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 complete degradation of diclofenac was achieved. According to obtained results, the photocatalytic decomposition by

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nanopowder mixture could be considered as a promising method for removal of dioclofenac. 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


Emerging photovoltaic technologies such as organic (OPV) or perovskite (PVK) 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 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 the 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 PVK. For our experiments, we considered disassembled OPV and PVK 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 PVK 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 PVK were leached out. The leached metals of the PVK 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. In all in all, the current and coming results give empirical insights of the emissions of future OPV and PVK application 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 C60, fullerenes are, accumulated to be expected 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 (t1/2) of the C60 was 13.1 days, with an overall degradation of 45.1% that was accompanied by the formation of functionalized C60-like structures. Furthermore, mass spectrometric analysis confirmed the presence of a large number of fullerenoid 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 (t1/2 of 4.7–6.4 days for silicon dioxide and 9.7 days for sands). However, small but consistent 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 (AgNO3), 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 (therefore 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 AgNO3 and that there was no difference in avoidance towards either chemical. At fluctuating temperatures, E. albidus avoided AgNPs at 20°C (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 AgNO3 than AgNPs. For the other treatments, there was no difference in Ag+ accumulation. Ag+ tissue contents at fluctuating temperatures were similar to the treatments exposed to the homologous treatments of AgNPs and AgNO3. 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
Ec-friendly profile of pegylated nanographene oxide synthesized for medical application

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Nanographene oxide (nGO) was recently exploited in the biological field for the development of new antitumoural 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 43°C. 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 assess 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, neomate 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 photophytic transparency of D. magna, the thermal fluctuation was simply tagged in gos by 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 (CeO2), 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 on the influence of environmental factors on the measurement a land dynamic 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 are observed. In addition to the characterisation the sorption of organic substances on nanoparticle surfaces are evaluated and quantified in terms of sorption isotherms
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 corrosion phenomena 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 antiomy nanoparticles (rGO-SnSps) 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 voltammetry and adsorptive stripping voltammetry. Further work aims to elucidate if adsorbed biomolecules onto nanoparticle surfaces are still accessible for microbial degradation. 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 a relatively small surface area, the glassy carbon electrode delivered almost the same application performances. The results therefore exhibited strong, stable and reliable cyclic and stripping voltammetric peak currents. The rGO-SnSps/SPCE sensor exhibited a detection limit of 0.45, 0.49 and 0.49 pg/L for Pt(B), Pt(II) and Rh(III) respectively. The rGO-SnSps/GCE sensor exhibited a detection limit of 0.42, 0.26 and 0.34 pg/L for Pt(B), Pt(II) and Rh(III) respectively. The differences and similarities in the results obtained are discussed in this paper.

**MO063 Concentrations of butyltin in Scottish marine sediments: improved detection using Isootope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS)**
K. Donald, C. Robinson, T. Low, Marine Scotland Science; J.J. García Alonso, University of Oviedo

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 Scottish beaches, TBT residues continue to be significant environmental hazards. Whilst recent studies on inimose in marine gastospods show that the degree of exposure to butyltins is decreasing, imposing due to organotin 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. MBS has recently improved analytical method performance by introducing the use of ID-GCMS quantitation in which mixture containing significant environmental hazards constituted 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 (90/690/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 pg/g dry 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**

The assessment of the ecotoxicological potential of titanium dioxide grades is progressing through the REACH evaluation process. Conventionally, the aquatic hazard assessment scheme is based on the subchronic 14-day static test with 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 different nano- and micro-TiO2 grades were investigated according to OECD Monograph No. 29: Guidance Document on Transformation / Dissolution (TD) of Metals and Metal Compounds in Aquatic 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 TiO2 grades in solution remain dispersed or produce ionic Ti species. The six different commercial TiO2 grades tested varied in nano-surface area and crystalline structure. Solid T/D studies were performed with a loading of 1 mg TiO2/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 polyleuthsulfenole 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 dissolvded 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-TiO2 grades tested were not able to 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**
M. Gutsch, H. Šillerová, Czech University of Life Sciences Prague / Geosciences

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 Guéguen et al. 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 re-dissolved with H2O and HNO3 to produce an oxidizing acidic mixture to aid in the dissolution of organic matrix complexes in the samples. Once dissolved, dissolved 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 H2O and HNO3 to remove the remaining organic matrix complexes associated with Ni. Samples were then basalified 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 H2O2 and HNO3, we were able to optimize purification. Removal of organic matter occurred successfully via the oxidizing acidic mixture, which is essential for analysis through thermal ionization mass spectrometry (TIMS). TIMS is not 100% efficient 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, H2O2 and HNO3) were essential for removal of other metals/organics and purification of Ni. On average, the separation and purification of Ni was achieved at 91 ± 8%. The results demonstrated our method to be efficient and useful for further work in analysis of Ni in the environment. 1. B. Guéguen, et al. Geostand. Geoanal. Res. 37. 297. 2013

**MO066 Influence of the lead anions to plant growth in artificial soil condition**
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ecological functions; V. Terekhova, Institute of Ecology and Evolution RAS / Lab Ecological Soil Functions

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 ventured is one of the general chemical properties that maximize 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 microplastics disposable plates. The soil contained 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 the cumulative mass of 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 intensive map of the metal concentrations at the community level was generated from 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 ± 2.6%) < Ni (19 ± 7%) < As (27 ± 7%) < Cu (36 ± 10%) < Zn (63 ± 13%) < Pb (82 ± 12%) < Cd (86 ± 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 potential for plant uptake of these metals. However, the overall hazard indices and carcinogenic risks, incorporating biota. The sub-sampling 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 “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 the addition of Nano-emeralds 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 metal emissions 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 of Cu, Cd, Pb and Zn 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 concentrations of Cr were in the mining waste while Pb and Zn showed the highest values in that affected by fertilizer and 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 and bioavailability of metals in soils and road dust.

MO069 Plant growth and changes in redox potential modulate the effects of biochar for improving wetland soils polluted by mine wastes


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 printing trees -BPT- and from sewage sludge -BSS-) and plant growth on Phragmites australis, Sphagnum fuscocreticosum, SJ 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-M+B+P; MW+B+P; MW+B+SS; MW+B+SS+P. Six different commercial nano- and micro-TiO2 grades at a loading of 1 mg TiO2/L of each grade at pH 6 were tested. The measurement of metal concentrations were not affected neither by the addition of biochar nor by the metal oxides solubilisation at lower Eh values. The presence of Pb contributed to mobilise these three metals towards the aqueous environment and to increase the concentration of Pb towards the aqueous environment and to increase the concentration of Pb towards the aqueous environment and to increase the concentration of Pb towards the aqueous environment and to increase the concentration of Phragmites australis, Sphagnum fuscocreticosum, SJ 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-M+B+P; MW+B+P; MW+B+SS; MW+B+SS+P. Six different commercial nano- and micro-TiO2 grades at a loading of 1 mg TiO2/L of each grade at pH 6 were tested. The measurement of metal concentrations were not affected neither by the addition of biochar nor by the metal oxides solubilisation at lower Eh values. The presence of Pb contributed to mobilise these three metals towards the aqueous environment and to increase the metal concentrations.

MO070 Derivation of soil threshold concentrations for arsenic, copper, and nickel: Results of chemical extraction methods


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 – plants”, there is no influence between total metal contents and their effects on microbes, plants and invertebrates. A realistic risk assessment of metals should preferentially consider bioavailability 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 bioavailability
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 oxynate arsenate. Then, next to the aqua regia extraction, metals will be extracted by different neutral salts (Ca(NO3)2 adjusted to soil ionic strength, 0.01 M CaCl2, and 1 M NH4NO3), chelating agent (DTPA/CaCl2), or weak acid (043 M HNO3). The extraction strength of the different methods follows the order HNO3 > DTPA > NH4NO3 > CaCl2 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 polluted 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 most toxic metal to human health and can directly cause damage to the respiratory system and reactive oxygen species (ROS) that might damage cell macromolecules, especially polyunsaturated lipids and proteins. With long-term irrigation containing Cd, Cd would accumulate in soil and then become accessible to plants and human, further causing health injury. Therefore this issue is highly needed being noticed. According to 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 46.6 and 96.3%. Results from the two-phase extraction showed that the proportion 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 extraction was positively correlated with sand and analysis was conducted with all the 18 soils. Based on PBET tests, it could observe that concentration of Cd in gastric extraction was positively correlated with sand and mobility of Cd in soil were evaluated through a sequential extraction procedure (SEP) 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.

MO073 Lanthanide distribution across environmental compartments in a LREE enriched geological area in Quebec (Canada)
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Predicting copper and zinc speciation in estuarine waters
C. Constantino, Atkins; S. Comber, Plymouth University / Environmental Science; E. Stravinskene, Siberian Federal University; Y. N. Grigoriev, Siberian Federal University / Department of Ecology and Environmental Study

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 is the dissolved oxygen concentration (DOC), which is an important factor because the DOC concentration of aquatic systems can be strongly related to the differences observed in the richness and abundance of plant species. We found that the specie Lamarkcia 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 soils than in the most contaminated soils. This implies that the accumulation of trace elements is strongly related to the differences observed in the richness and abundance of plant species. 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 unvegetated soils. Our results indicated that trace element bioavailability is strongly related to the differences observed in the richness and abundance of plant species. 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 unvegetated soils. 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MO075
Metabolism in sewage effluent
C. Constantino, Atkins; S. Comber, Plymouth University / Environmental Science;
M.D. Scrimshaw, Brunel University / Institute for the Environment
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 physical-chemical characteristics such as pH 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 varied considerably across samples indicating the presence of a range of complexing agents in sewage effluents.

MO076
Predicting copper and zinc speciation in estuarine waters
S. Comber, Plymouth University / Environmental Science; I.B. Pearson, Plymouth University / SoGeES; C.B. Braundgart, Plymouth University; P. Worsford, Plymouth University / Geography, Earth and Environmental Sciences
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 metal-specific toxicity to sensitive species. Some countries such as the UK have set new metal EQS for copper and zinc, with the copper standard based on an empirical relationship between copper toxicity to mussels (Mytilius sp.) and ambient dissolved organic carbon (DOC) concentrations. At low DOC concentrations the 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 WHO, UNEP, and USEPA. The study of the dissolved metal fraction is critical for understanding metal interactions and toxicity in estuarine habitats.

MO077
A pH-controlled flow-through system exposing fathead minnows and rainbow trout to copper under soft water conditions with added dissolved organic carbon
A.S. Cardwell, Oregon State University / Faculty Research Assistant; K. Delbeke, S. Baken, European Copper Institute; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology
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 

MO078
Effect of salinity on silver absorption in hemolymph of the marine amphipod (Parhyale hawaiensis)
M. Vannucci-Silva, UNICAMP; S. Cadore, Campinas State University; G. Umbuzeiro, SCHOOL OF TECHNOLOGY -UNICAMP / LEAL.
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, it 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 the relationship between salinity and silver absorption was the same across all the organisms.

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
D. Hug Peter, E. Castella, University of Geneva / Department FA Forel for Environmental and Aquatic Sciences; and Institute for Environmental Sciences; Y. Slaveykova, University of Geneva / Department FA. Forel of environmental and aquatic sciences
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 the connected secondary channel to the main channel is likely to induce 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 analysis. Additionally, the invertebrate community composition before and after the restoration was compared.

MO080
Metal complexation in sewage effluent
P. Scrimshaw, Brunel University / Institute for the Environment
Estuarine metal speciation data with that derived from available models including WHO, UNEP, and USEPA. The study of the dissolved metal fraction is critical for understanding metal interactions and toxicity in estuarine habitats.
MO080  Fate, speciation, and bioavailability of vanadium in sediments
S. Nedrich, University of Michigan; G. Burton, University of Michigan / School of Natural Resources & Environment
Relatively little is known about the bioavailability and speciation of vanadium (V) in the environment 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 (acidified), individual metals, total metal content, Fe-Mn-oxhydroxide 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 traps 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. The study showed that 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 vary greatly 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
C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering; W. Chen, Kaohsiung Medical University / Department of Biomedical Science and Environmental Biology
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 for Hg(II) and methylmercury (MeHg), respectively, compared with those in water with the range of 2.60x10⁻⁶ - 7.32x10⁻⁴ and 8.57x10⁻¹⁰ - 2.41x10⁻⁹ 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 dominant species in both water and sediment, accounting for nearly 85% 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/organ burdens would reach equilibrium before 180 days of exposure in all rivers. Among all exposed tissues/organs, kidney has the highest internal exposure doses of Hg(II) ranging from 0.021 - 0.135 µg g⁻¹ ww. In contrast, muscle had the lowest internal exposure doses of 0.0001 - 0.0003 µg 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 river study. 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
Y. van der Westhuizen, Western Cape University of Technology / Chemistry; B. Silwana, DURHAM UNIVERSITY; C. Walters, CSIR / Natural Resources and the Environment; C. Van der Horst, University of The Western Cape / SensorLab Department of Chemistry
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 exchanged harmful for the benthic community. The Berg River Catchment, 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, Fe²⁺, and filtered metal concentrations were determined. A new field method for assessing chromium and nickel pollution by the user. They replace complicated full-BLM procedures and increase the applicability for “routine” water type reporting laboratories. They are quick and easy of use. A new field method for assessing chromium and nickel pollution was determined by atomic absorption spectrophotometry (AAS). By soil digestion method with diacid, total Cr and Ni were analysed in the leachate and sediment. An empirical regression model was constructed with stones across the slopes thereby intercepting the surface runoff of the Plenthic gleysol and the Mollic gleysol (1) were relatively low, regardless of horizon and 5 samples at the bottom of the unsaturated zone. In each profiles, 5 samples were collected at surface (5 cm) and subsurface (up to 4 m) metal spatial distribution. However, the thresholds and underlying mechanisms cannot be considered in the same way -by-case – for different metals. In the absence of sediment contamination and under controlled conditions and to investigate the impact of metal mobilization on biotic ligand models (BLMs) are the standards are generic and therefore apply to all surface waters. The importance of these quality standards are developed to protect the ecosystem from adverse effects. These quality standards are further shown that aquatic environments have 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 shown that sediments with low levels of 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 biogeochemical distribution of trace metals in the Patumahoe River, Upland (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.
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, physiochemical parameters explain up to 97% of geochemically normalized second metal metal loadings (ions). In the field, only redox, pH and organic matter 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 ($p^2 = 0.42, r^2 < 0.001$). 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.

**MO087**

Bioavailable Eqs for Lead for European Surface Waters: An Update on DOC- and BLM-based Approaches

J. Chowdhury, International Lead Association / Senior Scientist - Environment; P. Verdonck, University of Antwerp / Professor of Marine Chemistry; F. Verdonck, F. Verdonck

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 sorption in freshwater, other parameters, specially water redox and pH, can 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 SSIDS (species sensitivity distributions) that were normalized with the chronic BLM, the calculated 5th percentile hazardous concentrations of Pb (i.e., ecological threshold concentration of 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 EQS 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 commonly 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 immobilization in soils of tropical urban lowlands

C. Defo, Indian Agricultural Research Institute / Water Science and Technology; Y.B. Fälmer Kiuban, FASADA University of Dschang / Agronomy and Soil Science

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 vertic zone) of heavy metal concentrations, regardless of the sampling station. The retention ratios (RR) of the Rhodic ferralsol, Xanthic ferralsol and Mollis gleysoy 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 retained values of Chromatic gleysoy and Mollis gleysoy (1) were relatively low, regardless of the heavy metal concerned. Therefore, RR was generally < 30%: about 10% for Plinthic gleysoy (in case of Cr); 20% for Mollis gleysoy (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 dumps to restrict the movement of heavy metals in the environment.

**MO089**

Principal component analysis for choosing of barrier materials for groundwater treatment

L. Chotin, Department of Chemistry, Umeå University / Department of Chemistry, L. Lövgren, Umea University / Department of Chemistry; M. Tyskling, Department of Chemistry Umeå University; P. Haglund, Umea University / Department of Chemistry

Industrial discharge of organic and inorganic pollutants has resulted in contaminated groundwater worldwide. As groundwater contaminants may have serious long-term consequences for groundwater remediation, it is vital to find the right combination of sorbents to immobilize them. This study was conducted to determine the sorption behavior of certain organic and inorganic contaminants on adsorption media. A survey was conducted to assess the presence and concentrations of carbon tetrachloride, benzene, toluene, and ethylbenzene in contaminated groundwater. Methane, ethane, ethylene, and carbon dioxide were also detected in the groundwater. The results showed that the predominant contaminants in the groundwater were carbon tetrachloride, benzene, toluene, and ethylbenzene. The study also indicated that methane, ethane, ethylene, and carbon dioxide were present in the groundwater. The study concluded that the presence of these contaminants in the groundwater has significant implications for the remediation of the contaminated site. The study recommended the use of a combination of adsorption media, including activated carbon, clinoptilolite, and zeolite, to effectively remove the contaminants from the groundwater. These media were effective in removing carbon tetrachloride, benzene, toluene, and ethylbenzene from the groundwater. The study also recommended the use of a combination of biological and physical-chemical treatments to further improve the remediation of the contaminated site. The study concluded that the combination of sorbents is effective in removing the contaminants from the groundwater, and the use of a combination of biological and physical-chemical treatments is effective in further improving the remediation of the contaminated site.
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 Substances Society). The DOC concentration was 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 PC1 and PC2, it can be seen that some samples that make the greatest contribution to the creation of the first principal component (PC1) are the sorbents that remove organic pollutants. The greatest contribution to the second principal component (PC2) 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 M. Yildiz, Akdeniz University; C. Moral, Akdeniz University / Environmental Engineering

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 glucuronic 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 alginate 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 Cu²⁺/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, Cd²⁺, Cu²⁺ and Pb²⁺, 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.


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 consider the case of lead as an essential element, 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 order to determine the best one based on the concentration of the most toxic Pb. After that, we tested the effect of some of the selected mineral supplements on Pb bioaccessibility in two different soils. Results showed that Pb bioaccessibility of 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 bioaccessibility 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 levels. After that, we evaluated the effect of some of the plots was performed 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. We showed that blood Pb levels in sheep exposed to mineral supplementation decreased throughout the experiment while Pb levels in blood of sheep not supplemented. Mineral supplements enriched with Ca, P, Mn, Mg, I and Cu would potentially decrease Pb bioavailability and be used as a palitative cost-effective measure to reduce Pb exposure of livestock and wildlife in Pb contaminated areas.

MO092 Bioaccessibility of selected trace elements in indoor dust from Istanbul-Turkey P.B. Kurt Karakus, Bursa Technical University / Environmental Engineering Department; K. Akdogan, U.C.L.M.-C.S.I.C. / Instituto de Investigacion en Recursos Continentales, CNRS UMR 7360; G. Echevarria, University of Lorraine; L. Continente, CNRS UMR 7360

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 glucuronic 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 alginate 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 Cu²⁺/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, Cd²⁺, Cu²⁺ and Pb²⁺, 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.
daily intake (PMTDI) and the dietary intake reference (DRI) levels for Cu are 50–
500 mg kg⁻¹ BW and 700–900 mg kg⁻¹ BW, respectively and it seems that the
estimated daily intake (EDI) of Cu (0.007 mg 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 ultralimnic shoreline of Lake Ohrid (Albania)
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Interdisciplinaire des Environnements Continentaux, CNRS UMR 7360; P.
Rousselle, D.A. Vignati, University of Lorraine / Laboratoire Interdisciplinaire des
Environnements Continentaux, CNRS UMR 7360; S. Devín, LIEC - Université de
Lorraine - CNRS / Laboratoire Interdisciplinaire des Environnements
Continentaux, CNRS UMR 7360; G. Echevarria, University of Lorraine; L.
Giamberini, Université de Lorraine, CNRS UMR 7360 / Laboratoire
Interdisciplinaire des Environnements Continentaux, CNRS UMR 7360; F.
Guéroud, University of Lorraine / Laboratoire Interdisciplinaire des
Environnements Continentaux, CNRS UMR 7360

Lake's located in mining areas can be heavily contaminated by metals, which
afterwards may be accumulated in tissues of aquatic organisms and transferred up
the 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 across ultramafic areas, also contribute to metal inputs into
the lake. As aquaculture production is rather extensive in the lake, 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 Ohiroid 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 matrix
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Trade elements; N. Waegeoneers, CODA-CERVA / OD Chemical Safety of the Food
Chain; K. Cheyns, CODA-CERVA / OD Chemical Safety of the Food Chain
Trade elements; A. Rutten, CODA-CERVA / Trade Element Service
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 (Asₐ), inorganic arsenic (Asᵢ), AB (arsenobetaine), DMA (dimethyl arsenate) and MA (monomethyl arsenate). As specification analysis was conducted by
HPLC-ICP-MS, by means of a method which is commonly used for As speciation
analysis in food (eef 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 As. The results
discovered that bioaccessibility of Asᵢ 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 As fraction. The FS matrix was taken to 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. Risks related to the intake of As species were evaluated by comparing the calculated exposure to the reference values suggested by
ATSDR(2007), IJCEFA(2011) and EPSA(2009). If the reference value was a MRL, the
reference value was the MRL value. If the reference value was a BMDL value, then a
'Margin-of-exposure' (MOE=BMDL/exposure) was calculated. The conclusion
'concern' was given when the MOE< 100. In relation to As 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 As 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 metal contaminants
in contaminated soils
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ECOSYS; D. Vile, INRA / UMR 7399 LEPS; F. VANDENBULCKE, INRA / Université de
EA Université Libre de Bruxelles / Eco-systemes Terrestres Anthropisés Cité Scientifique SN Rde F Villeneuve
d’Ascq France; F. Gimbert, University of Franche-Comté / UMR ChronoEnvironment; M. Hedde, INRA / UMR ECOSYS

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 (i.e. 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
chemical metal extractions, 2) environmental bioavailability: metal absorption
by the organism, measured by internal metal contents, 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 datasets
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 waters
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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
resource. However, the supply of good quality water becoming increasingly difficult in view of increasing global pollution levels, demand for water, contamination, high operational costs of water treatment and high 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-
and silver-impregnated chitosan beads has been successfully performed. In this study,
bimetallic iron-silver nanoparticles were 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 its efficiency in the
removal of Cd(II) from wastewaters was found to be 91.4%. Furthermore, the

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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 on the effect of biochar on the sorption-desorption of pesticides. A pseudo-steady state with soil and biochar-soil systems at 10% (w/w). Based on the partition coefficient \( K_{d} \) 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 \( K_{d} \) values were 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 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.

MO099 Glucose mineralization in soils of contrasting textures under application of S-metolachlor, terbutylazine, and mesotrione alone and in a mixture.

K.F. MENDES, University of São Paulo USP / Laboratory of Ecotoxicology; S.A. Colleran, ESALQ USP / Agronomy; R.F. Pimpinato, Universidade de São Paulo, Centro de Energia Nuclear na Agricultura; v. tornisielo, CENA Center for Nuclear Energy in Agriculture.

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, terbutylazine, 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 the results with applied herbicides (alone 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.

G. Spickermann, ADAMA Deutschland GmbH; S. Roulier, ADAMA

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 pH2. 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, such as daily minimum 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, data of soil dissipation of two sites at模仿 meteorological 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 EFSAS (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 rates based on measured meteorological and 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 ecosystems. In this project different pollutants were detected. 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 been developed through IMPACTA project. Previous information is available about the distribution of PAHs and organochlorinated compounds in these 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 (terbutylazine, propazine, etc), insecticides (chlorpyrifos, methyl-chlorpyrifos, etc), personal care products (galaxolide, tricolosan, 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 homogeneous and the most significant peaks were found in the low 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\textregistered tool to automate PEC\textsubscript{sw} calculations, extract data from the models TOXSWA and SWAN and create MS Word\textsuperscript{TM} tables in the current DRR format. Solution 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 presently uses template with predefined 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\textsubscript{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\textsubscript{sw} at Step4 ’Step4ward’ 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 recession 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 connects the information in a temporary VR-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 ‘Step4ward’ 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 sediment retention index (as a measure for the adsorbed 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 present, with the poster will discuss and present 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)

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 at the same measurement times in the same measurement sequences, 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.), carp 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 from pesticides applied in nearby fields. The modeled results from monitoring data also reveal impacts of transport processes related to long-range transport.

MO105 H2O-ot 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 paper introduces the H2O-ot 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 (BKS0) 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 (BE). After a dataset as a result of the 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, lemmna, and chironomus) as Exposure-Toxicity-Ratios. For the aquatic risk evaluation acute risk on aquatic organisms is not a determinant for farmers' decisions on its pesticide use. To change this, we believe that online tools such as the H2O-ot 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 - GAP-S v 1.1
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Water-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 EFSA 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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Groundwater exposure assessment scenarios for plant protection products can be imitated using sequestration 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 representation 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 the PEARL-Hamburg 2D hydrogeochemical model 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 stream 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. 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 field-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 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 (PGS) and dedicated edge-of-field monitoring studies. This approach has proved useful establishing 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 groundwater 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 water, 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, 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 QuiChERS 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 area where to implement risk mitigation measures. For instance, in the last few years, the monitoring frameworks for 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 underevaluated 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 all. The 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 substance, and narrowed water systems 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 cm soil layer. 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 map and the spatial 95th percentile of the PEC in soil within the region is calculated directly on resulting datasets. VULPES was used by the risk managers to evaluate 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 environmental active substances, while considering their robust assessment by regulators.
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 a 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 data are rare, however that transport of pesticides can be influenced by the potential transport of autumn applied pesticides from soil to water during winter and spring 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 profiles. The results from the experiment in the transport of 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.

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 use 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 some specific situations or 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/m³. 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, air samples were collected for 14 days with an active sampler. Results of CUPs concentrations in ambient air will be presented. Moreover, spatial and temporal variations will be discussed.

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 specific exposure data. 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.

Simultaneous air and water passive sampler modelling with ethylene-vinyl acetate: theory and experiment

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We apply a multiphase 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 measured partition ratios, we are able to calculate concentrations at a downstream monitoring well were calculated. The simulation and solute transport simulations carried out using OpenGeoSys, in which resulting upper boundary conditions for the subsequent two-dimensional groundwater flow model were provided by means of a sophisticated Time of Flight Modelling in Support of Monitoring Studies model. This approach has been applied as a case study at the whole territory. However, monitoring of pesticides is a challenging task because of the variable characteristics of the pesticides as well as the different resistance profiles. Therefore, there is a need to develop suitable exposure assessment methods that can be applied under these conditions. In this context, passive sampling methods have been proposed as an alternative approach to assess the exposure of people to pesticides. The aim of this study was to develop a methodological approach to assess the exposure of people to pesticides using passive air and water samplers. The method is based on the measurement of the concentration of pesticides in the air and water samples collected using passive samplers. The method is validated using a spiked environmental matrix and results are compared with those obtained using active sampling methods. The method is found to be suitable for the assessment of pesticide exposure in the environment and can be used to monitor the exposure of people to pesticides. 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 specific exposure data. 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.
MO119 Integrated risk assessment of pesticide, the use of MERLIN-Expo tool for combining exposure assessment with toxicological effects on the food web

MO120 Good agricultural practice (GAP) in the light of meteorological constraints

MO121 Modelling the fate of pesticides in European and French cropping systems: Incorporation of complex agricultural practices in the regulatory risk assessment

MO122 New software development for automated environmental fate modeling and reporting

MO123 Detection of CeO2 (nano) Particles in Sewage Sludge

found to range from 365-899 mg/L and 513-899 mg/L 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 Kd and Koc also follow the sequence Linuron > Diuron, which is the same order for their Koc. 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 1/s values reported in the literature, and the Koc 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.

MO122 New software development for automated environmental fate modeling and reporting

R. Juraska, T. Hauck, Dr Knoll Consulting GmbH

For the registration of plant protection products the agrochemical industry needs to provide environmental risk assessment 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, parameterized, or run separately, which unfortunately is time-consuming and labor-intensive. 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 input errors and reduce the effort required for quality control.

MO123 Detection of CeO2 (nano) Particles in Sewage Sludge

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The results of the study will also identify the presence of 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) CeO₂ particles in the sewage sludge. Concentrations of cerium (Ce) and lanthanum (La) were quantified in acid digested sewage 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 whereas the precipitate was used for transmission electron microscopy (2700cx, 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 varied on a comparable scale. Although the natural background concentrations of both elements are very variable, the ratio of Ce:La reported from soils is mostly around 2:1. Engineered CeO₂-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 analyses whereby the sludge indeed contained anthropogenic CeO₂-NP. Due to their high density, CeO₂ particles appeared as very bright particles in high angular annular dark field images, which greatly facilitated their detection. The size of the CeO₂ particles ranged from ~50 – 200 nm. EDX analysis of selected CeO₂ particles revealed significant La concentrations in some of the CeO₂ particles, but also showed CeO₂ particles without La. These ‘pure’ CeO₂-NP points towards an anthropogenic source of CeO₂-Particles. Additional TEM analysis of individual CeO₂ – NP in combination with single particle ICP-MS analysis was used to quantify the CeO₂-NP abundances and to distinguish between natural and anthropogenic (nano) CeO₂ particles in the sewage sludge.

MOI24
Toxicity of silver nanoparticles on two Lemna spp.: effects on photosynthesis and growth
C. Pinheiro, University of Aveiro; I. Lopes, University of Aveiro / Department of Biology and CESAM, 3810-193 Aveiro, C. Venâncio, Department of Biology / Biology, S. Francisco, University of Aveiro / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Biology 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 physiological endpoints (frount 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 front 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 further generate knowledge about the toxicity of AgNPs to aquatic macrophytes but also to underline the need of considering the sensitivity differences between species when examining the risks of AgNPs to the aquatic environment. Keywords: nanomaterial, aquatic plant, chlorophyll, TWEEN 20

MOI25
Toxicity assessment of four phosphorus adsorbents used for lake restoration
M. Salcedo, University of Granada / Ecology; A. Baun, DTU Environment / DTU Environment; I. De Vicente Álvarez-Manzaneda, University of Granada / Ecology 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 phosphate lacking due to the trophication 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 CEA Silver nanoparticles as well as commercial magnetic and a commercial micrionized 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 was 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 g l⁻¹. The results show that the adsorbents concentrations was 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 g l⁻¹. The results show that the adsorbents concentrations was 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 g l⁻¹.
The toxicity assessment of engineered nanoparticles in algal bioassays A. Asanova, Krasnoyarsk State Agricultural University / Institute of agro-ecological technologies; V. Polonskiy, Krasnoyarsk State Agrarian University; Y. S. Grigorev, Siberian Federal University / Department of Ecology and Environmental Study

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 in the exponential-phase. Strains of the Chlorella vulgaris Beijer were cultivated at the 36ºC 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 (TiO2) with nominal particle size of 100-190 nm and of silica nanoparticles (SiO2) 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 inhibition concentration EC50, which causes a 50% reduction of RIDF and optical density to the control. As the main effect we considered that the greatest inhibitory effect on the culture of Chlorella vulgaris Beijer was caused by Ag nanoparticles – the EC50 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 TiO2 nanoparticles reduce the rate of growth and photosynthetic cell function of algae insignificantly. It was conducted that the more SiO2 nanoparticles size was the greater the toxic effect was. Therefore, the value of EC50 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 SiO2 nanoparticles with particle size of 10-15 nm showed no toxic effect at all.

Iron oxide nanoparticles effects on Daphnia magna: Implications for environmental risks and ecological impacts V. Gonzalez, V. Gonzalez - Andres, Leitat Technological Center; C. Delpivo, LEITAT; S. Vázquez-Camps, LEITAT / Human & Environmental Health & Safety

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 as environmental remediation agent to remove 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. Different nanoparticles have different functionalization, 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 (EC50 > 900 mg/L), mainly related with the physical interactions of the humic acids, OAD 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.

Effects of silver nanoparticles from effluents of wastewater treatment plants (WWTPs) on the behaviour of Daphnia magna S. Hartmann, University of Siegen, Institute of Biology / Institute of Biology; K. P wrest, University of Siegen / Institute of Biology; L. Kugler, University of Siegen / Institute of Biology

Over the last ten years the amount of manufactured nanomaterials (MMNs) has been increasing in the environment due to their use in products such textiles, cosmetics, sunscreens, and paints. MMNs accumulate in the aquatic ecosystem after passing wastewater treatment plants. The remaining MMN levels may cause increased toxicity for the aquatic environment. One of the most abundant MMNs 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-MMN and Ag-NPs from effluents from WWTP we focus on daphnia as a key species in a typical freshwater food web. The effect of Ag-NP on organism which could be relevance for their toxicity and risk assessment. Daphnia magna adults from the effluent did not significantly alter the measured parameters comparatively to the control effluent.
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.

R. Louch, University of Siegen / Institute of Biology; S. Hartmann, University of Siegen, Institute of Biology / Institute of Biology; K. Witte, University of Siegen / Institute of Biology

Silver nanoparticles (Ag-NPs) are increasingly becoming an environmental concern due to their widespread use in many products such as textiles, cosmetics, waterproofings 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 (1.25g/L, 2.5g/L, 5.0g/L and 10.0g/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 hermaphrodite freshwater snail Physa acuta was chosen as a non-target aquatic organism to evaluate the potential toxic effects of AgNPs (as pristine and Ag-Ss) due to their vital role in the aquatic ecosystem as efficient grazers and detritivores. Acute tests (96h) with juveniles (<1 mm) 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-S 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 Material Effects of Silver Nanocolloids on Fish Reproduction using Medaka

M. Katsuka, Toyo University / Life Sciences; Y. Kato, Toyo University; S. Kawamura, Toyo University / Research Center for Life and Environmental Sciences

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 deal with uncomfortable environment. Early life stage of fish (eggs 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 main organ and cell effects are associated to reproduction and 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 acclimated in each 1 L of embryo rearing medium (ERM) under co-exposure conditions at 25 ± 0.5°C 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 acclamation, 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 80.2 ± 3.5 (%) in 0.5 mg/L and 34.9 ± 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 antibiotic system and histopathology on gills and kidneys of fish, Prochilodus lineatus

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Iron oxide nanoparticles effects on Daphnia magna: Implications for engineered nanomaterials to the environment. Our experiments were carried out on model systems (NM300K) and Ag-NPs from WWTP (NM300K) and Ag-NPs from effluent of wastewater treatment plants (WWTP) and Ag-NP contaminated algae affect reproduction and mortality in Daphnia magna over multiple generations. The aim of this study was to determine 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 (1.25g/L, 2.5g/L, 5.0g/L and 10.0g/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.

The present study investigated the toxicity of titanium dioxide nanoparticles (NP-TiO2) 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-TiO2 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-TiO2; GSH levels increased after exposure to NP-TiO2 concentrations higher than 1.5 mg L-1. Hyperplasia, 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 hyper trophy 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-TiO2 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, main epithelial cell hypertrophy, main epithelial cell hypertrophy 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. The results showed that 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 Cd²⁺, (2) MWCNT and medium RPMI containing 10% of fetal bovine serum (FBS) for 30 minutes and thereafter Cd²⁺, and (3) MWCNT and Cd²⁺ for 30 minutes and then RPMI and 10% FSB medium. Cytotoxic assays were performed using Trypan Blue, Neutral Red, LDH leakage, MTCNT and apoptosis and necrosis rates. Morphological analysis and cell survival assays were also performed. This study evidenced that the order of compounds on the interaction MWCNT and Cd²⁺ interfere on the cytotoxicological 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 intervenes on cadmium toxicity. The presence of cadmium 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 dye the increased formation of endosomes and/or enlargement of lysosomes. Further researches on standardization methods and reproducible and effective protocols for evaluating the cytotoxic activity of nanoparticles, 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 AgNO₃, as a metal salt reference, in three standard soils (OECD, RefeSol 01-A, heterogeneous nanoparticles) was evident. This effect was more pronounced and more consistent for AgNP than for AgNO₃ 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 and F. candida reproduction 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 comparative 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 associated to explain the data generated using 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 (R² mostly > 90%) and internal robustness (Q² and Q²cv 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 R² values above 90% and the interpretation of real experimental variation between prediction limits 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 variables 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 mathematical 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


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 ecosystems. In this context, a microcosm 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 (DT₅₀=11 d) following a half-life of 11 days in the P2 (DT₅₀= 17 d), although 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 assessed 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 J. Liu, Firmenich Inc / Research Development; M. Emberger, Firmenich, Inc; S. Gimeno, Firmenich / Protective Safety and Regulatory Affairs 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 Myrrhne® Neo and indirect photodegradation of Pamplewood will be presented. Direct photodegradation quantum yield of Myrrhne® Neo was determined to be 0.097 ± 0.009 in the laboratory using PNA/pyridine as a solvent. Direct photodegradation half-lives of Myrrhne® 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 LCM-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 xOH was the main reactive intermediate responsible for indirect photodegradation, resulting in a Pamplewood half-life of about 1.6 days in a single cycle. Based on this, a second-order rate constant (8.48 x 10⁻⁴ M⁻¹ s⁻¹) for photodegradation products were studied by GC-MS whereby intermediates of Pamplewood continued to photodegrade. Complete mineralization was observed for 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 polycaprolactone dieol exposed to different wastewater environments D. Rivas, CSIC/IDEA/E / Environmental Chemistry; B. Zonja, IDEA-CSIC / Environmental Chemistry; S. Perez, IDEA-CSIC; A. Ginebreda, CSIC / Spanish National Research Council / Department of Environmental Sciences; M. Costa, CSIC-IDEA; D. Barceló, Catalan Institute for Water Research ICRA / Department of Environmental Chemistry MALDI-IMAGING MS (FAB-MS) consists of 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 in the development of adapted microorganisms communities. In this study, an advanced wastewater treatment plant was evaluated and 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, 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. Acknowledgements This work has been supported by the European Communities 7th Framework Programme funding under Grant Agreement No. 603629-ENV-2013-6.2.1-Globaqua. This work has also been supported by the Generalitat de Catalunya (Consolidated Research Groups “2014 SGR 418 - Water and Soil Quality Unit”).

MO146 Regulatory assessment of environmental persistence: OECD biodegradability screening tests – need for improvements and possibilities for enhancements S. Gimeno, F. Candida, T. Junker, Firmenich / Protective Safety and Regulatory Affairs 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 assessment 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 UV/VCB sub-stances) are addressed. Within a 2-(half)-days’ workshop and expert discussion of different verite expert groups and representatives from different insitutions (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)"Assessment of environmental persistence: regulatory requirements and practical possibilities – available test systems, identification of technical constraints and recommendations for future testing". SETAC Europe 27th Annual Meeting Abstract Book.
MO147  
Realistic to improve the OECD 306 marine biodegradation screening test  
A. Ott, T. Martin, Newcastle University / School of Civil Engineering and Geosciences; G. Whale, Shell Health / Shell Health Risk Science Team; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; B. Rowles, Cefas / OCNS; R.J. Davenport, Newcastle University / School of Civil Engineering and Geosciences 
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 tier 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 closest 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  
A. Lai Shuen Lyn, ETH Zürich; C. Eiwag / Institute of Biogeochemistry and Pollutant Dynamics; D. Latino, Eiwag Swiss Federal Institute of Aquatic Science and Technology / Department of Environmental Chemistry; K. Fenner, ETH Zürich 
Microbial biotransformation is the primary route of degradation for most pesticides in soils. Eiwag-SOIL is an unprecedentedly rich database package of envIPpath 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; envIPpath.org). Biotransforming compounds, besides their metabolic values containing 4716 non-redundant extended 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. 

MO149  
The effect of aging seawater on microbial communities before preforming biodegradation tests according to OECD test guideline 306  
A. Wenneberg, NIVA - Norwegian Institute for Water Research / Systems, engineering and technology; A. Lillegaard, NIVA - Norwegian Institute for Water Research 
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. The hypothesis was 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 performed in small test bottles, and then the lag-phase of the test will be shorter. This study was performed 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 shortened and the test was improved for 10% and gave 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 soluable, 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 UV/C. 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 in simulating environmental conditions. Consistency with screening test results and formation of Non Extractable Residues will be covered as well as the inhibitory properties of new testing approaches 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  
S. Fiebig, H. Mäischak, Noack Laboratorien GmbH; C. Maess, DR.U.NOACK-LABORATORIEN 
Within persistence 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 the test 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 designs, of which the OECD 301 A/D and E, respectively, in principle resemble the methods from TG OECD 306. The method OECD 301E (Manometric Respirometry) is another 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 C. Lowrig, Charles River / Environmental Fate and Metabolism 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 sediment 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 provide 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 B. A. Poersat, University of Amsterdam/IBED Institute / Institut voor biodiversiteit en ecosystem dynamiek; M. Bruijnis, VU University Amsterdam; R. Helmus, University of Amsterdam / IBED; J.A. Roling, R.J. van Spanning, VU University Amsterdam; P. de Voogt, J. Parsons, University of Amsterdam / IBED. 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. The OECD (2004) has proposed that inorganic carbon should be added to the medium of the inoculum. This addition may facilitate adaptation and result in a better estimation of the environmental fate 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 different conditions. The biodegradation capacity of the inoculum measured in RBTs 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 differences in the bacterial community structure of the culture. This will 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 and biodegradation of isopyrazam 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, Vernicom AG; M. Seyfried, Firmenich. The biodegradation properties of the carbamate insecticide isopyrazam (WWTs) has been included 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 about the ways this 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 into the 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 competitive 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 degree of coverage (95-98%). In our hands, samples for FISH preparations originating from a WWTP from a rural area of Geneva (La Villette / 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
C. Sweetlove, IOREAL SA / Research and Innovation; J. Chenèble, IOREAL Research; Y. Barthel, Eurofins Expertises Environnementales / Eurofins Expertises Environnementales; M. Meletta Denat, D. Brockmann, INRA; E. M. L. Alves, IOREAL Research & Innovation / Life Sciences Direction; T. Gerald, University of Nantes / Microbiology
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
T. Wagner, J. Parsons, University of Amsterdam / IBED; A. Langenhoff, Wageningen University / Department of Environmental Technology; H. Rijnaarts, Wageningen University / Subdepartment of Environmental Technology; P. de Voogt, University of Amsterdam / IBED
Cooling towers account for a significant part of the industrial fresh water uptake. Several treatment technologies like reverse osmosis, electrodeionization and membrane distillation are utilized to facilitate the reuse of discharged brackish cooling tower water. Next to a moderate salinity of 4 g/L, cooling tower 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 biodegradation processes in the inoculum and cooling tower water. 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 degradation of the substances? - How important is the 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
J. Wee, Korea University / Division of Environmental Science and Ecological Engineering; Y. Lee, Y. Kim, Korea University; J. Son, Korea University / School of Chemical and Biological Engineering; H. Moon, Korea University / Institute of Environmental Health and Natural Resources; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering; J. Wee, Korea University / Division of Environmental Science and Ecological Engineering; J. Son, Korea University / School of Chemical and Biological Engineering; H. Moon, Korea University / Institute of Environmental Health and Natural Resources; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering; J. Wee, Korea University / Division of Environmental Science and Ecological Engineering; J. Son, Korea University / School of Chemical and Biological Engineering; H. Moon, Korea University / Institute of Environmental Health and Natural Resources; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering
Glucaraldehyde (biocide), PEG (surfactant) and HEDP (antiscalant). The following non-specific, comprehensive eubacterial probe. Thanks to this monitoring incubation phase of individual tests, particularly after test compound was added.

MO160
The environmental fate of 14C-POEA in water-sediment systems
M.R. Shepard, Monsanto Co / Fate and Metabolism, Environmental Science; M. Kurtzweil, Monsanto Company; G. von Merey, Monsanto Europe S.A. / Regulatory
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. 14C-Labeled POEA was synthesized for use in the study to aid in analysis of the surfactant, to assess mineralization to CO2, and to allow for mass balance determinations. POEA dissipated rapidly from the water column with a DT50 of 2 to 3 hours through a combination of metabolism and adsorption to sediment. Metabolism was extensive as evidenced by the formation of 14CO2 ranging from 10 to 15% in the two water treatment systems. A total recovery of all metabolites (including unreleased POEA) was achieved using LC-MS and confirmed using a derivatization approach. Two main classes were identified that consist of tertiary amine metabolites containing both polyoxyethylene glycol and carboxylic acids (PFPEs) 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
H. Birch, DTU Environment / Department of Environmental Engineering; H. A. Andersen, Technical University of Denmark / Department of Environmental Engineering; M. Comber, Mike Comber Consulting; P. Mayer, Technical University of Denmark / Department of Environmental Engineering
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 considering 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 to 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_w, were up to 72
times higher than test system biodegradation rate constants, k_eff. True water
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
R. Janssen, Technical University of Denmark / Department of Environmental
Engineering; H. Birch, DTU Environment / Department of Environmental
Engineering; P. Mayer, Technical University of Denmark / Department of
Environmental Engineering
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.

MO163 Quantification of sub ppt levels of per- and polyfluoralkyl substances in various
waters utilizing advanced ultra-high performance liquid chromatography and triple
duplo quadrupole mass spectrometry
P. Abrahamsson, M. Chadaia, Agilent Technologies; L. Ahrens, Swedish University
of Agricultural Sciences / Dept. of Aquatic Sciences and Assessment
A method for the determination of per- and polyfluoralkyl substances (PFAs) 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 PFAs including 4
perfluoralkyl sulfonates (PFAS) (PFBA, PFHxS, PFOS, PFDoS), 13
perfluoralkyl carboxylates (PFCA) (PFBA, PFFA, PFHxNa, PFPoA, PFNA,
PFDA, PFHxDA, PFDoDA, PFFDA, PFHxDA, PFDoDa), 3
perfluorooctane sulfonamides (FOSA, MeFOSA, EfFOSA), 2
perfluorooctane sulfonamideethenols (FOSNE) (MeFOSO2, EfFOSO2), 3
perfluorooctane sulfonamidoacetacids (FOSA-SA) (FOSA-SA, MeFOSA-SA,
EfFOSA-SA) and 6:2 fluorotelomer carboxylate (6:2 FTCAs). 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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IDAAE; M. Ferrag, IDAAE-CSIC / Environmental Chemistry; D. Barcelo,
IRQAB-CSIC / Dept Environmental Chemistry
Key words: Fluorotelomer carboxylic acids (FTCAs); environmental samples;
GC-MS/MS. Abstract A great effort has been carried out to assess the occurrence of
poly- and perfluoralkyl substances (PFAS) 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 physical-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 pretreatment was based on
alkaline digestion for FTCAs extraction and in-column assistance for the detection of
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
BF3-MeOH (14%) and analyzed by GC-MS/MS. The chromatographic separation
was carried out using TRACE TR-5M column by TRACER Ultra gas
chromatograph (ThermoFisher Scientific). The detection was carried out with a
coupled triple quadrupole mass spectrometer TSQ QuantumTM Access Max
(ThermoFisher Scientific) using electrop 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
T. Anumol, Agilent; S. Dagnino, Imperial College / Department of Chemical
Environmental Engineering ChEE; S.A. Snyder, University of Arizona / Chemical
and Environmental Engineering
Due to their unique chemical properties, poly- and perfluoralkyl substances
(PFAS) have been historically chosen as a cleaning agent 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
fluorooctanoic acid (PFNA) and perfluoronic acid (PFNA), may have health implications in
humans. Therefore, PFNA and PFOS among others have been phased out, and
regulated in the EU, US and other countries. However, with the reduction in usage of
PFNA and other PFCA, several alternative polyfluorinated compounds such as
fluorotelomer acids and di-substituted polyfluoralkyl 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 PFCA (C4 to C9) was monitored using an LC/MS-MS. Results show,
the 6:2 FTUCA transformed into the 6-C PFCA (PFHxSA) 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 traces of other PFCA formed and
suggests that a n=2 FTUCA chieffy degrades to an n=3 PFOA on oxygen
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 PFCA formed were lower With the increased use of
ozone and UV-hydrogen peroxide AOP in water treatment, especially in potable
water treatment, these data suggest that increased concentrations of PFCA may result
in finished drinking water.

MO167 Non-invasive Samples as a Biomarker of Human Bio-monitoring to
Perfluoralkyl substances (PFASs)
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Engineering; H. Jeong, Pusan National University; G. Song, Pohang University of
Science and Technology / Department of Environmental Science and
Engineering; J. Oh, Pusan National University / Department of Civil and Environmental
Engineering
Perfluoralkyl substances (PFAs), 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 Board and potential
endocrine disruptor. Many researches have been examined the PFAS 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
these treatments. Individual sensitive 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, 0.058 to 2.24 ng/mL in urine, and 0.284 to 3.92 ng/mL 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 matrices (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? Y. COSTAUX, C. BACH, C. ROSIN, J. MUNOZ, Y. 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 acid (PFASs) used in these 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 fluorotoluenes (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 (PFCAs) in great tits (Parus major) along a pollution gradient in Antwerp, Belgium, and their effects on reproduction. T. Groffen, Systemic Physiological and Ecotoxicological Research (SHERE), University of Antwerp / Biology; A. López Antia, Universiteit Antwerpen / Biology; L. Bertrand, University of Antwerp / Biology
Perfluorinated compounds (PFCAs) have been produced for over five decades. Due to their hydrophobic and lipophobic character they are suitable for a wide range of applications. However, PFCAs may enter the environment, accumulate in wildlife and may cause detrimental effects. The widespread use of PFCAs 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 PFCAs (8 perfluorooalkyl carboxylic acids (PFCAs) and 4 perfluorooalkyl sulfonic acids (PFSA)) in non-destructive samples of a terrestrial songbird, the great tit, at a fluorochrome chemical plant in Antwerp, Belgium. In addition, samples from four other areas were collected, representing a gradient in distance from the pollution source. At each site, serum and hair samples were collected. Furthermore, reproductive success at each area has been assessed and related to PFCAs concentrations in these eggs. The PFOS and perfluorodecane sulfonate (PFDS) concentrations measured at the site of the fluorochrome plant are among the highest ever reported in wildlife with median concentrations of 3425 ng/g for PFOS and 3938 ng/g for PFDS. The results indicate that even at moderate 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 PFCAs 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 third solution can only be quantified, to investigate temporal changes of PFCAs concentrations using non-destructive
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 (catase, superoxide dismutase, glutathione-S-transferase and glutathione peroxidase), biotransformation (cytochrome P450A1 and lipid homoeostasis (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 pharmacokinetics 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. PFHxA elimination is excreted via 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 toxicity hazard assessment and compares the results to reported exposure concentrations to characterize potential human exposure risk. The key mammalian toxicity 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 then from a historic long-chain concentration (HEC) in hair. This HEC has then been 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 impervious to water (aka water repellent) and to prevent water-based 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 footprints. 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 criteria have framed the criteria for selecting a suitable DWR finish and what the current new technologies are.

MO176 New Concave / NICOLE technical report on the environmental fate and effects of PFAS, remedial options and legislative developments
T. Pancras, H. Slenders, ARCADIS; B. Beuthe, BP; J. Smith, Shell Global Solutions Int; M. Spence, CONCAWE / Water, Soil and Waste; L. Ross, ARCADIS; T. Albergaria, Instituto Superior de Engenharia do Porto, Instituto Politécnico do Porto
A recent report commissioned by the European Petroleum Refiners research division (Concave) 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 (PFASs), 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 firefighting 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 (persistence, bioaccumulative and toxic) for humans and wildlife, which has led to low regulatory limits for these compounds. Moreover, as the class of PFAS substances increases, restrictions are being extended to other PFAS compounds that have the potential to degrade to 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 Concave 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 Perfluorooxyalkyl Substances
R. Buck, The Chemours Company / Fluoroproducts; S. Korzeniowski, BeachEdge Consulting; W. Lehrenbaum, Crowell & Moring; M. Malvasi, Solvay Specialty Polymers
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 fluoroalcohol on nematodes - Comparison of single-species and community responses
S. Hoss, Ecoqua / Animal Ecology; A. Hagerbäumer, Bielefeld University / Animal Ecology; I. Roessink, Alterra / Environmental Risk Assessment Team; W. Traunspurger, University of Bielefeld / Department of Animal Ecology
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 (e.g. 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 fluoroalcohol (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 10435:2006), in aqueous and spiked sediment exposure. Moreover, nematode communities, sampled from outdoor microcosms 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 reported 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 protective effectiveness for nematode communities.

MO179  
Comparing the chemical and ecotoxicological persistence of sediment associated lufenuron  
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M. Kraak, University of Amsterdam / Department of Aquatic Environmental Ecology;  
J. Vonk, University of Amsterdam IBED Institute / Aquatic Environmental Ecology;  
I. Roessink, Alterra / Environmental Risk Assessment Team;  
T. Brock, Alterra, Wageningen University and Research Centre / Environmental Risk Assessment Team  
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 aging processes. Therefore the aim of the present project was to study the impact of age 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 to determine the chemical toxicity 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 aging 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 aging conditions. Two experiments yielded significantly different LC50-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 LC50 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 aging. This was indicated by the five-fold increase of the LC50-value for lufenuron after aging 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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S. Abel, University of Eastern Finland / Department of environmental and biological sciences;  
M. Leppinen, Finnish Environment Institute SYKE;  
M. Rasilainen, University of Jyvaskyla / Department of biological and Environmental Science;  
T. Oksanen, S. Hietaketo, J. Akkanen, University of Eastern Finland / Department of environmental and biological sciences  
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 site of these sediments was 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 (Lumbricus variegatus, OECD) and non-biting midge (Chironomus riparius, OECD). In addition, the usability of standard small 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 test did not give reliable results, since the EC50 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 reference lake with highest pH. 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 aeriation. For C. riparius, the required oxygen level (> 60%) was not reached without aeriation and they did not survive the low pH. L. stagnalis was not as sensitive to pH changes as other organisms. These experiments showed the difficulties with toxicity tests are used for metal-contaminated sediments. Besides metals, water chemistry and other substances (e.g. SO4, 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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D. B. Vignati, CNR SETAC Sediments Interest Group;  
C. N. LIEU UMR7360; S. Valsecchi, Water Research Institute - Italian National Research Council  
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 oxides 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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B.S. Martincigh, University of KwaZulu-Natal / Analytical and Environmental Chemistry;  
G. Birungi, Mbarara University of Science and Technology / Chemistry  
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 control measurements 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
MO184
Integrated ecotoxicological and epidemiological approach to evaluate the impact of Tamiflu metabolites on reproduction of medaka (Oryzias latipes). W. Chen, Y. Wu, M. Leong, Kaohsiung Medical University / Dept Biomedical Science and Environmental Biology

The purpose of this study is assessing the effects of environmental relevant Tamiflu metabolite concentrations on long-term survival, growth and reproduction of Taiwan medaka (Oryzias latipes). Firstly, this study to predict the environmental Tamiflu metabolite concentration from the perspective of age-specific influenza infected population and treatment dosages by epidemiological models. The study was carried out on a total of 1000 medaka in 10 aquariums with Tamiflu metabolite concentrations predicted to be environmentally relevant. Tamiflu metabolite concentration was observed to improve survival and growth energy budget. In a further study, 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 affect survival and growth energy budget of medaka. However, if the pandemic influenza outbreak induced higher than 300 μg L⁻¹ Tamiflu metabolite concentrations, 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

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 (ARV’s) therapy. Over the last three years, ARV’s have been detected throughout South African surface and drinking waters. Nevirapine is one of the first-line HIV ARVs which is prescribed to newborns, 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, 25.37 and 47.03 μg L⁻¹), 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 included feeding, swimming and schooling behaviour, survival, growth and development (length and weight). A histological assessment was carried out 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. A. Herrmann, Bayer Pharma AG; R. Laenge, Bayer AG / Dept Experimental Toxicology; M. Segura-Lepe, M. Keck, T. Sieger-Hartmann, Bayer Pharma AG

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 endpoints are needed expanding the 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 pharmacological 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 endpoints in both, embryos and fish, at the same time showing a clear interspecies effect. Furthermore, a subset analysis revealed amikacin induced metabolic perturbations specific for fish and fish embryos as well as shared maker candidates, which were consistent with expected metabolic changes of aminoglycoside induced renal toxicity in mammals. Further studies using compounds with different pharmacological activities are ongoing to provide a more comprehensive understanding of the capabilities 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. J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; K. Hutchinson, AstraZeneca Global Environment; L. Gunnarsson, University of Exeter / Biosciences College of Life and Environmental Sciences.

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 presence of these PAHs in the sediment samples is influenced by multiple factors, such as the source of the pollution and the environmental conditions. The environmental risk assessments (ERAs) estimated the potential environmental impact of these PAHs on the environment, using different endpoints, such as the risk quotient (RQ), which is calculated as the ratio of the measured concentration to a background concentration. The results obtained indicate that the RQs for the identified PAHs varied significantly, with some compounds showing very low RQs, indicating low risk, while others showed high RQs, indicating high risk. The study also showed that the use of different endpoints and the incorporation of additional factors, such as the fate and transport of the compounds, can significantly affect the risk assessment results.

MO188
pH-Dependence of Bacterial Toxicity: Role of Spectation for Ionizable Pharmaceuticals. A. Baumer, Helmholtz Centre for Environmental Research GmbH - UFZ; K. Bittermann, Helmholtz Centre for Environmental Research GmbH - UFZ / Analytical Environmental Chemistry; N. Klüver, Helmholtz centre for environmental research - UFZ / Department of Cell Toxicology; B. Escher, Helmholtz Centre for Environmental Research – UFZ / Toxicology.

Toxic 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 Q SAR 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). Essential and baseline toxicity Q SAR models are mostly based on the octanol-water partition coefficient Kow, simply by a linear relationship of log EPC and log Kow. The domain of applicability of most baseline toxicity Q SARs comprises neutral organic chemicals ranging from log Kow of approximately 1 to 7. About 80-70% of all APIs are ionizable 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 Q SAR development, e.g. by using the pH-dependent liposome water distribution ratio Dwn(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 pro-drug type agents 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 Kow or Dwn(pH) in baseline toxicity Q SARs by the Dwn(pH) many APIs will be classified as baseline toxicants. We investigated the pH dependence of cytotoxicity between pH 5.5 and 9. 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.
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 require 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, beclomiasonate, 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 Kow (R=0.002), weaker correlation to Log S (R=0.24) and molecular weight (R=0.23), and a stronger correlation to Log D (R=0.57), and pKa (R=0.56). The strength of correlation of Log S (R<0.83) and pKa (R=0.91) was significantly impacted by the (macro)enzymes reserpine 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.

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 lipophlicity. 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 (SxEREG-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 ml 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 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.

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 “PIE” (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.
Long-term effects of the anti-epileptic drug carbamazepine in Chironomus riparius - two multi-generation studies
K. Heye, Goethe University Frankfurt/Main / Aquatic Toxicology; J. Oehlmann, Johann Wolfgang Goethe Frankfurt / Aquatic Ecotoxicology

Midges like Chironomus riparius are mobile organisms, which can either be exposed to chemicals continuously for several generations or populated on uncontaminated habitats. Especially for contaminants like the anti-epileptic drug carbamazepine (CBZ) that is emitted throughout the entire year, the investigation of multiple generations is mandatory to provide reliable information on the development of the environment. In the current study we aim to investigate i) whether the response to long-term exposure changes within three subsequent generations (F0, F1, F2) and ii) whether future unexposed generations are affected by the pre-exposure of the parental generation (F0). 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 F0. 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 (F1 and F2) and to the same concentrations as in F0. In parallel, a recovery experiment was conducted, where larvae were transferred into unexposed medium in F0 and F1. As endpoints, mortalities 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 these midges to repeated exposure in the environment was increased again in F0. As these results were similar for pre-exposed midges that were transferred in unexposed medium, selection of less sensitive midges in F1 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 a direct derived from 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 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 100 ng/L are shown below: almesartan (571 ng/L), valsartan (405 ng/L), ibersartan (162 ng/L), candesartan (113 ng/L), losartan (117 ng/L) for antihypertensive agent, and sulpiride (546 ng/L) for antipsychotic, clorothyramycin (445 ng/L) for antibiotic agent, ketoprofen (150 ng/L) for analgesic antipyretic, bezafibrate (200 ng/L) for hyperlipidemia treatment drug, crotamiton (845 ng/L) for antipruritic. Among target ingredients, the detection concentration of active ingredient in medicines for the lifetime of their cases tends to be higher. The concentration 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 dissipated 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 rosuvastatin. This research is supported by the Research on Regulatory Science of Pharmaceuticals and Medical Devices from Japan Agency for Medical Research and development, AMED. 
P. Meriläinen, National Institute for Health and Welfare; N. Perkola, Finnish Environment Institute / Laboratory Centre

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 uncertain or unproven. A key health effect of some 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 artificial ground water production site that uses pumped river water as raw water. Selected emerging contaminants, including pharmaceuticals and artificial sweeteners, and perfluoralkyl acids (PFAA) were measured from water samples with an isolate 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 drinking water in the Turku region. 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 illnesses 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 ascesulfame. In exposure assessment daily intake of contaminants via orally consumed tap water was estimated and inhabitants were exposed for ibuprofen, acnesulfame and caffeine in noticeable amounts via drinking. Nevertheless when comparing to the chemicals' ADI values, none of the chemicals exceeded ADI and the calculated ADI proving the health risk is not significant. The artificial ground water do not cause any harm to human health in current regulatory perspective. However, some of the chemicals such as some PFAS 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.

MO201

From PEC-based trigger to a reasonable decision tree: Revision 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 (EMAP/CHMP/007/00, rev. 1) suggests to perform a pre-assessment when the concentration of a pharmaceutical in the environment is above 0.01 μg/L. The aim of this study was to review the "pre-assessment" scheme defined in the current EMAP guideline in the context of the revised EMA guideline (EMAP/CHMP/007/00, rev. 2) in December 2006. The phased approach consists 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 toxicity 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.

MO199

Hazard assessment of human pharmaceuticals - a review on data persistence, bioaccumulation, and toxicity

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Hazard assessment of persistence, bioaccumulation and toxicity is a central part of environmental risk assessment for chemicals in surface and ground water used for drinking water production.
MO203
E. Nfon, Smithers Viscent

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: PhA2E (Pharmaceutical Assessment and Transport Evaluation) model and the Geo-referenced Regional Exposure Assessment Tool for European Rivers (GREAT-ER). PhA2E is commonly used in the US and the exposure estimates predicted by PhA2E™ generally had a good fit with measured data from the USQ5. 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 micropolutants. 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 vary 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/L, MCPA = 80854 mg/L), whereas some pesticides showed a clear weekly application pattern (e.g. pethoxamid, metolachlor, terbutylazine 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 operated as a separate sewer system, strong water dilution of 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 of the incoming contaminants that were removed in water (OW), 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 wastes. The removal factors for OW 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 OW 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 particular 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, such as agricultural run-off and effluents, 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.SPF.984087) and IIH44009 Project.

MO208 Persistent pollutant that estimator tool: predicting recovery from legacy contamination in rivers

E. Nicol, C. Constantino, Atkins; M. Gardner, Atkins Ltd. 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 may be discharged unmodified to the aquatic environment. 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 development, the model may be applied to a range of scenarios for different control options for 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, 105, 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 (0.0085 pg/g), a reduction in BDE input of 35% 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 types in the environment. Possible scenarios for other trace contaminants will be presented.

MO209 Biological effects measures and adverse outcome pathways applied to effluent assessment

R. J. Brown, wca consulting; G. Merrington, WCA Environment Limited; D. Leverrett, WCA-Environment Ltd; S. Marsden-Jones, wca-consulting 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 approach still requires development. To evaluate the application of 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 value 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 potential misinterpretation of results may accumulate in downstream sediment. An AOP approach should be considered 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 carboamazepine

M. Pablos, INIA National Institute for Agricultural and Food Research and Technology; J. Martinez-Guitarte, UNED / Fisica Matematica y de Fluidos; M. Jimenez, The Complutense University / Histology Laboratory, Faculty of Veterinary Medicine; P. Garcia, INIA National Institute for Agricultural and Food Research and Technology / Department of Environment; A. FernAndez, INIA National Institute for Agricultural and Food Research and Technology / Laboratory for Ecotoxicology. Department of Environment; E.M. BeltrCe, INIA - National Institute for Agricultural and Food Research and Technology / Environment; M. Torrijos, INIA National Institute for Agricultural and Food Research and Technology / Department of Environment Laboratory for Ecotoxicology; C. FernCndez, INIA National Institute for Agricultural and Food Research and Technology / Department of Environment 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 reasons 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 carboamazepine at concentrations 100 and 1000 higher than the mean levels observed in reclaimed waters. Carboamazepine 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 related to potential thyroid effects were studied. The results are discussed. This work has been funded by the Spanish Ministry of Economy, Industry and Competitiveness 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 D. Gabbia, V. Pascoli, University of Padova / Department of Pharmaceutical and Pharmacological Sciences; G. Monteleone, W. Tiler, Eco-research; M. Carrara, University of Padova / Department of Pharmaceutical and Pharmacological Sciences 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 wastewater samples, measuring both primary nonspecific toxicity (cytotoxicity and ROS production), as well as markers of inflammation (IL-6 production) and antiobiotic metabolism (modification of CYP1A1 and CYP3A4 expressions). Chemical analysis for two wastewater samples revealed the presence of several drugs, including paracetamol, salbutamol, atenolol, ranitidine, sulfamethoxazole, omeprazole, carboamazepine, 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. In contrast with 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

P. Leonards, VU University, Institute for Environmental Studies / Department Environment and Health; J. Postma, Ecofide; T. Jonkers, VU University / Institute for Environmental Studies; M. Spence, CONCAWE / Water, Soil and Waste; G. Whale, Shell Health / Shell Health Risk Science Team As part of ongoing 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. The main pollutants in effluent include metals, hydrocarbons acting via narciss, 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 hydrocarbons might be relatively direct, novel approaches to analytical 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 their individual fractions, were performed in the event of finding a novel 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)
T.O. Soyebamu, UNIVERSITY OF LAGOS / Zoology; A.A. Sosanwo, University of Lagos Nigeria / Dept of Zoology
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 tested 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 LC50) of the effluents to P. reticulata was 71.50 mL/L (7.15%). Histological alterations observed included 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-dichlorohydrate, 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
A. Gerhardt, LimCo International GmbH
Micropollutants, such as pharmaceuticals, biocides and pesticides, can 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 wastewater treatment technologies and to detect and 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 (impact control). The content volume of the reactor was monitored using a fluorescence technology 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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Activities have a significant potential to bring the ecoinvent database closer to the origin. The ecoinvent database originates from anaerobic digestion of sewage sludge and therefore the global environmental impact is approximately 50% from anaerobic digestion of sewage sludge and 50% from anaerobic digestion of manure. While, detailed process information and thanks to that, to increase the confidence in the water reuse schemes for both public authorities and population and authorities. From this starting point, the focus of this study is to determine the environmental benefits and impacts of the reuse scheme implemented in the city of Sabadell by using Human Health Risk Assessment (HHRA), Life Cycle Assessment (LCA) and Water Impact Index (WIXX) methodologies. With this approach, the aim was to show that the reuse scheme is not a threat to public health and environment and 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 as 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 GMGIO data**

A. Haus, Institute of Environmental Engineering, ETH Zurich / Chair of Ecological Systems Design; S. Pfister, ETHZ / Chair of Ecological Systems Design

**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 system 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 (GMGIO) 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 both regions is available, ecoinvent is an average of 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 consumes biogas. Trade of biogas between 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. EIO database structures do however 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 case study, biorefinery work was done to add a preliminary hybridized LCA model to account for the uncertainty in our hybridised LCA output. To quantify the uncertainty for future scenarios that are being derived from this hybridised inventory, the uncertainties of the elements in the technosphere and biosphere have to be specified. For the elements coming from the EIO database and the flow model, data distributions are assumed, with a mean given by the parameter’s value and a standard deviation of 10%. 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 software is still under development. As the source system is planned to be derived using the ABM, we do now investigate the results of individual biorefineries. Hybridised LCA models have the potential to reduce the truncation error, yet the lack of quantitative uncertainty measures for EIO databases still poses some concern. While our implementation of uncertainty quantification can contribute by giving a mean 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 GHG 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 combines the advantages of both Life Cycle Impact Assessment 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 through 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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Many financial institution are starting to engage in sustainability-related activities, 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 deliver net positive carbon outcomes. It will show how to integrate planetary boundaries as well as address calls to correct LCA developments and products can deliver net positive carbon outcomes. The approach was developed as a way to measure and to integrate the impacts on the biodiversity. For the first issue the methods used are simple and pragmatic data collection with informative visualization of the results. Future work will focus on the 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 case study, biorefinery work was done to add a preliminary hybridized LCA model to account for the uncertainty in our hybridised LCA output. To quantify the uncertainty for future scenarios that are being derived from this hybridised inventory, the uncertainties of the elements in the technosphere and biosphere have to be specified. For the elements coming from the EIO database and the flow model, data distributions are assumed, with a mean given by the parameter’s value and a standard deviation of 10%. 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 software is still under development. As the source system is planned to be derived using the ABM, we do now investigate the results of individual biorefineries. Hybridised LCA models have the potential to reduce the truncation error, yet the lack of quantitative uncertainty measures for EIO databases still poses some concern. While our implementation of uncertainty quantification can contribute by giving a mean 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.
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 a accepted and robust method that is used for both the assessment and the quantification of environmental impacts.

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 regional, 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 that can be used for regional accounting for the material consumption and waste flows in the BCR. The material flows shall not only be presented as an 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 sources 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, Product Data). Nevertheless, the lack of detailed and robust information about the environmental footprint of the construction sector remains a major challenge and a limit to any environmental impact assessment. The aim of this work is to develop and validate a methodology for LCA upscaling 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 method uses 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 biodiversiy and on graph theory for the in-situ impacts due to the habitat fragmentation. DImOSim (District MODéller 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 calculation of the habitat fragmentation impacts on the biodiversity. The EVE platform - B IM and citygal workflow allowed the authors to support the integration 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 lands, 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 LCA 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 (LCB) concept. These include exemplars vital for establishing how developments and products can deliver net positive carbon outcomes. It will show how to integrate planetary occupation and transformation flows for the 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 LCA 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.
adoption, 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 (LCBA) which LCBA metrics are being proposed how LCBA is being used now for products and projects. The need for beyond LCA measures in order to find out the derivation of life cycle 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
C. Batancourt Quirga, Ingeniería e Instituto Universidad Nacional Autónoma de México / Engineering Institute; E. Citernero, Ingeniería e Instituto Universidad Nacional Autónoma de México / Environmental Engineering
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 further places from urban centers, which has generated environmental 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 included the assessment 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 age and type of motor vehicle, and the water consumption. The use phase of the houses was divided in the following stages: transport, construction, operation and maintenance. 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 follows: passenger car transport contributed to human toxicity, freshwater eutrophication, fossil depletion and climate change. Energy consumption contributed to eutrophication of fresh water, human toxicity and climate change. Bus transportation contributed 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
T. Opher, A. Shapira, E. Friedler, Technion - Israel Institute of Technology
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 treatment is one of the widely accepted and 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 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 wastewater 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 alternative scenarios address 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 confirmed 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 order to derive the final results of the assessment. A strong agreement among experts 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, SETAC Environment Europe)
M. Amores Barrero, M. Calvet, CETaqua Water Technology Centre; D. Marin-Navarro, CETaqua Water Technology Centre / Environment and Socioeconomics; M. Termes, CETAQUA; M. Salamero, Aigües de Barcelona; Y. Lorenzo-Toja, CETaqua, Water Technology Centre; R. Bellido, Ajuntament de Sant Feliu de Llobregat
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 provided the first step for implementing the circular economy concept in a region for emerge opportunities between actors through input and output flows analysis (water, energy, materials) to shift linear economy model (take – make – dispose) for circular economy model (close the loops). SFL 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 reusing rainwater 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 involving 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
Y. Xie, Nanjing Institute of Environmental Sciences of the Ministry of Environmental Protection
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. Based on water environmental effects analysis tool, strip types of contributing zones with different buffer radii were focused on quantitative relationships between land use pattern and water quality in Argun River basin of Sino Russian border area. The Circular Economy Package of the European Commission comprises of an action plan covering the entire cycle from creation, utilization to waste management. However, some barriers were identified involving the Legal Framework as understanding applicable legal issues, rights and obligations of public administrations and private companies is essential for implementing a circular economy. 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.

MO231
Circular economy: Recycling glass fibre reinforced composites (GRP)
G. Girardi, M. Caporaso, C. Giardini, L. Tamburini, C. Lazzari,�E. Ferri, Università degli Studi di Palermo / Department of Industrial Engineering; M. Ferrua, Università degli Studi di Palermo / Department of Chemical Engineering; N. Jonkers, Ecochain Technologies / chain management; T. Beentjes, J. Mos, Ecochain Technologies
The concept of circular economy requires a paradigm shift in the way that products are designed and manufactured. Material flows analysis (water, energy, materials) to shift linear economy model (take – make – dispose) for circular economy model (close the loops). Circular economy “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.
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 (TDN, kg N).’ 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 270m³/day and 2.5 kg of NH₄⁺ concentration per m³ 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 LCA 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-site ammonium 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 NH₄⁺-N concentrations, while the SHARON-Anammox is less CC impacting under lower NH₄⁺-N concentrations. When analyzed from a circular economy perspective, the NAR system was revealed as being able to economically supporting 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 Y. Otsu, University of Tokyo; N. Isubo, Tokyo City University According to IPCC 4th 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 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(10000US$), loss is 1.6E+08(10000US$) and CC is 6.6E+07(10000US$). As a rate of total damage and loss, Thailand is 70% and 60%. Others occurred 30% and 40%, but almost rate occurred Asia or Europe. By analyzing these results are expected to know each country/sector risk.

**Life Cycle Data and Modeling Challenges: solutions (P)**

MO233 Circular economy: Recycling glass fibre reinforced composites (GRP) according to EN 15804 Module D (End-of-Life) through applied LCA scenarios V. Vlaminckx, HOBAS

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 reprocessing 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 NOx 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, MD) according to EN12000 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. N. Jonkers, Ecochain Technologies / chain management; T. Beentjes, J. Mos., Ecochain Technologies The traditional approach of creating separate LCA reports for single products has some 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 E. edahiri, N. Isubo, Tokyo City University 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 indoor and outdoor 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 verify the reliability and availability of our results.

MO236 Review schemes and reviewers’ selection criteria for the Life Cycle Data Network S. Fejzo, EC-JRC 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 quick reviewed 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/OEFP) scheme is adding further compliance rules. The aim of this paper is to define the eligibility rules for reviewers, in the different schemes. At the meeting in 2011 the LCI/ILCD and ILCD readymade LCIA life cycle initiative, was released the “Global Guidance Principles (GPP) for Life Cycle Assessment Databases” The document provides also details on review that were further 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 a platform to find info about reviewers and reviewer teams. The eligibility 3 different schemes (ILCD, ILCD EL, and PEF/RE) the tool can be expanded also for the GPP 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. Different schemes have different minimum score to be reached, in order to be eligible or 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 it fulfills 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.

MO239 Exploring prospective scenarios of water supply mix

S.O. Leelö, IRSTEA Montpellier / UMR-ITAP ELSA; P. Roux, Irstea / UMR ITAP ELSA (ELSA-PACT); M. Nüñez, IRSTEA Montpellier; E. Liseau, IRSTEA Montpellier / UMR ITAP ELSA; R.K. Rosenbaum, National Research Institute of Science and Technology for Environment and Agriculture - Irstea / UMR ITAP ELSA. 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 life cycle inventory data and the LCI data on human health, ecosystem quality, and natural resources as part of the life cycle impact assessment (LCIA) step. Their contribution to considering uncertainty in LCIA. These best practices and state of the art will be presented and the uncertainties in LCA to improve current practice. Feedback is encouraged.

MO237 Roadmap toward addressing and communicating uncertainty in LCA

L. Laurin, EarthShift Global; B.W. Vigon, Brevaca Environmental Consulting LLC; P. Fanke, Technical University of Denmark / Quantitative Sustainability Assessment (QSA) Division. Life Cycle Assessment (LCA) is a tool that aims at helping decision makers to make environmentally sustainable choices. 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 areas of: 1) characterization of uncertainty, 2) state of the art, 3) hypothesis, 4) variability and uncertainty, 5) communication of uncertainty, 6) uncertainty and LCA decision-making, 7) tools to support uncertainty assessment, 8) best practices for communicating and reporting uncertainty, and 9) gaps and barriers to implementation. 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 used by using only available data in Ecoinvent for corn and wheat cultures. For the conversion steps, literature data have been used along with some industrial data. Based on these data, it is possible to conclude that the production of 1 kg of glucose for three different cases. The results underline that the differences between the two databases are smaller than the differences between specific data (Belgian 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 and the GaBi database have been modeled 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 sensitivity of the results to parameters not controlled by the LCA practitioner, such as the datasets hypotheses, the software differences, etc.
used to develop similar models for other background processes.

Higher risk, assessment and management of natural toxins: state-of-the-art, challenges and future perspectives (P)

MO241 Ecoinvent and GaBi models are readily available in two databases remain high. The presentation will underline where these differences are specific data (Belgium data) or non-specific data (Ecoinvent) for the agricultural (yield, amount of fertilizers, etc.), either using EcoInvent or Gabi datasets (MO238).

Agricultural Research Centre (CRA-W) (2014) have been used as primary data for preliminary results underline the high impact of the agricultural step, so a special MO239 report uncertainty in LCA to improve current practice. Feedback is encouraged.

A.L. MERCHAN, University of Liege / Chemical Engineering, PEPs; A. Leonard, University of Liège - Chemical Engineering / Dpt of Chemical Engineering - PEPs; N. Meron, Tel Aviv University; V. Blass, Tel-Aviv University / The Coller School of Management.

MO242 MO242 Quantification of the carcinogen ptaquiloside in complex matrices D. Lindqvist, L. Raumus, Metropolitan University College (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 noseyquiperep glycocisde, 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-pterinosin. 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 pterosin 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 deglycosylated (1Ml PTA soln. was added 75g/L 1 M NaOH, heated for 1hr (35°C)), cooled to room temperature and added 75µL acid (tripplicate; 1.0M H2SO4, 1.0M HCl, 0.2M HNO3, 1.0M FA, 1.0M HBr and HCl). PTA and pterosin B was quantified by LC-MS. Deglycosylation 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-pterinosin. Prior to any new studies using HPLC results only in estimation of 60% 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-pterinosin indicates possible severe artefacts in such methods. Studies using indirect techniques should therefore be treated with caution and older studies in order to address the same data gaps identified during the peer review of the ecotoxicological risk assessment (MO243).

MO243 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 intermediary risks. Enclosures set up: an old system as new semi-field approach for common voles to assess their risk on plant protection products F. Blanckenhagen, J. Ludwigs, C. Clausen, B. Ziesemann, Rifcon GmbH 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 reasons classical refined approach 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

K. Kyriakopoulou, I. Kandris, I. Pachiti, K.M. Kasiotis, A. Spyropoulou, K. Machera, Benaki Phytopathological Institute; A. Santourian, M. Glossiotis, G. Papakostis, A. Tzifakis, S. Georgakopoulos, A. Ippolito, EFSA - European Food Safety Authority / Pesticides Unit

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, work and 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 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 72 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 regions inside and outside Europe (France, Italy, Spain, UK, USA, etc) for different crops (P. tanacetifolia, grapes, raspessed, 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, DT50 and DT90 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

M. Ortiz Santaliestra, Institute for Environmental Sciences University of Koblenz-Landau / Institute for Environmental Sciences; F. Mougeot, C.S.I.C; V. Alcaide, IRIAF / Centro de Investigación Agroambiental El Chaparillo; M. Martinez-Haro, IREC / Instituto de Investigación en Recursos Científicos / Departamento de Líneas de Investigación, K. Arnold, University of York / Environment Research; R. Mateo, U.C.L.M.-C.S.I.C / Instituto de Investigacion en Recursos Cientificos 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 validated in Spain, France and Cyprus. 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 environmental management business. Its timeliness is fundamental 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 on the classification, authorization and renewed registration 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 the 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 and 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 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 value to risk assessment and risk management. Under Regulation (EC) 1107/2009, it is increasingly difficult to demonstrate that a substance poses no risk. 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 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


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 fulfill the intended ecological function, a minimum standard in the protection from entries of agrochemicals such as pesticides would be required. However, the regulation does not 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 posterior will clarify the legal environment 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 PPI 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 in the field, and the OECD to produce a guide for the implementation 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_{WR} for R scenarios was determined, as well as the percent reduction of these components of the PEC_{WR} 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 each 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
A. Vanden Bosch, ARCHE, I. Huygens, Arche consulting

Geraniol is a terpenoid compound present in many essential oils. It is used as a 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 Bicidal 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 within the different legislations, a comparison of the timelines for review/rewaival of active substance dossier and we highlight differences in approaches for exposure and risk assessments. The aim is to elucidate where consistent 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?

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 log K_{OW} for structurally similar compounds. 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 (NOAEC). The NOAEC represents a concentration at which aquatic communities will experience adverse effects but can reach steady state. However, the acceptance of these endpoints 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 waterbodies too small to be considered representative of real waters. In this study, microcosms were used to study the impact of waterbodies affected areas upstream 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 their abundance levels must be considered, and both a conservative yet realistic approach to deriving Reliable Endpoints from Tricky Taxa 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 assessing ‘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, zooplankton. Intended fludioxonil concentrations of the spiked sediment were control - solvent control – 60 – 180 – 540 – 1620 – 4860 – 14580 µg 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 overlying water and provides more detailed information on endpoints with the help of MDDs can be straightforward when analysing highly abundant taxa with a clear monotonous dose response. However, for taxa with low or no 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 assessing ‘tricky taxa’ to reduce the subjectivity that may affect the endpoints of PPPs.
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 pulse 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
K. Raatsl-Hooper, P. Havens, Dow AgroSciences LLC; G. Mereghalli, Dow AgroSciences Italia srl / Environmental Regulatory Sciences Ecotoxicology Exposure and effects assessment elements: a review. 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 hazard assessment was performed on a single herbicide and the distribution of water quality benchmark concentrations (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
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 study 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 (towards the end of the study), 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 chemicals to produce more realistic exposure scenarios whilst reducing environmental stress.

MO257
Statistical power in higher tier studies. How much is enough?
J. Howells, J. Ashford, F. Pickering, Cambridge Environmental Assessments
Statistical power in higher tier studies. How much is enough? (test protocols.) This contribution performs statistical power analyses for several different sets of its ‘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 the design of urban blue infrastructures, or 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
The role of ecotoxicology in fisheries science and aquaculture (P)

MO261

Halogenated and organophosphorus flame retardants in aquaculture samples (Aznar-Almeny), IDAECA CSIC; y. aminiot, University of Plymouth; J. Viñ-A-Cano, IDAECA CSIC; M. K.-ck-Schulmeyer, IDAECA CSIC / Water and Soil Quality Research Group Dept of Environmental Chemistry; J. Readman, University of Plymouth; A. Marques, L. Godinho, IPMA IP; E. Botton, Aesforia SL; F. Ferrari, AEIFICORIA; V. I. Botu, University of Ioannina; T. Albanis, University of Ioannina / Department of Chemistry; A. Kringspedt, NIVa Norwegian Institute for Water Research; E. Aljarrat, IDAECA-CSIC / Department of Environmental Chemistry; D. Barcelo, IQAB-CSIC / Dept Environmental Chemistry

Flame retardants (FRs) are compounds applied to materials to increase their fire resistance. Polymorbidiphenyl 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. 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 plasticizers. The use of OPFRs was 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 PEBB), 5 dechloranes (syn- and anti-Dechlorane Plus and Deds 602, 603 and 604) and 8 OPFRs. For the sample preparation freeze-dried samples were used for water and internal standards were used. HR extracts were analysed by GC-MS. Method recoveries ranged from 76% to 93% 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 ranged from 76-112% and LODs and LOQs were 0.02/1.25 ng/g dw and 0.005-4.44 ng/g dw, respectively. All groups of compounds were detected in 75-100% of samples for each matrix, except for OPFRs 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.

MO262

Biotransformation of clofibric acid in zebrafish embryos (Danio rerio) as determined by liquid chromatography-high resolution-mass spectrometry (S. Brox, B. Seiwert, N. Haase, Helmholtz Centre for Environmental Research UEFZ / Department of Analytical Chemistry; T. Reentsma, Helmholtz Centre for Environmental Research / Department of Analytical Chemistry; E. Küster, Helmholtz Centre for Environmental Research, Dept.Bioanalytical Ecotoxicology / Bioanalytical Ecotoxicology

Biotransformation of clofibric acid by the 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 glucuronate acid, and, reported here for the first time, with carbonate, taurine, and aminochloroanisole. These new aryl-containing compounds were identified using non-target screening that may represent a new detoxification pathway. Sulfate containing TPs occurred already after 3h of exposure (7hbpf), and from 48h of exposure (52hpf) onwards, all TPs were detected. The detection of these TPs indicates not only the first 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 in terms of a possible alternative to species used in toxicological studies.

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

TUU001

An intestinal fish cell barrier model to assess absorption of poorly soluble organic chemicals in vitro (H. E. Eawag - Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; F. Begnud, Firmenich / DRAP; C. Debonnelle; Firmenich; F. Berthaud, Firmenich / Research and Development; S. Gimeno, Firmenich / Product Safety and Regulatory Affairs; K. Schirmer, Eawag / Environmental Toxicology

Fish, like other aquatic organisms, accumulate organic chemicals present in water (biocentrization) 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 transport of poorly soluble chemicals, such as some fragrance compounds. From an experimental point of view, working with fragrances poses certain difficulties due to their hydropobicity (logKow > 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 Damascena beta as example. The permeation of Damascena 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 with a driving force of permeation mass balance. A full recovery of Damascena 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 Damascena beta

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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 RTG-GC was successfully used to measure the permeation of the fragment Damasonce beta. A specific ex vivo chamber system was used to study permeation in 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.

TU/002 Effects of repeated exposures to fine particles (PM10-18.2-5.5) and ultrafine particles (PM10.18) on inflammatory chronic lung diseases exacerbation J. Setty, G. Garçon, Université de Lille 2 / EA IMPECS; F. Denayer, Université de Lille 2 / EA IMPECS; J. F. Allorge, Université de Lille 2 / EA IMPECS; L. Renard, Université de Lille 2 / EA IMPECS; L. Canivet, Université de Lille 2 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 more easily in the respiratory tract, where pulmonary clearance is less effective. There have been numerous experimental studies demonstrating that fine airborne particles (PM2.5) through oxidative stress-induced airway inflammation, epigenetic modifications or cell cycle alteration, however, a few of studies have paid close attention to the ultrafine fraction (PM0.18), which can represent more than 70% of total number of particles. Particles also attain new properties at nanometric scale. Because of their high surface area and small size they are likely to be more 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 PM16.5 and PM0.18. 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 oxidative stress at the cellular level to lung tissues. Characterized and differentiated 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. PM16.5 treatment trigger a more accentuate response than PM2.5, 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.

TU/003 New alternative carcinogenic prediction assay using flatworm stem cell proliferation patterns A. Stevens, Hasselt University / Centre for Environmental Sciences; M. Willems, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; M. Pluissant, J. Floem, E. Winkelmanns, Hasselt University / Centre for Environmental Sciences; T. Artois, Hasselt University; K. Smets, Hasselt University / Centre for Environmental Sciences 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 carcinogenicity which is important for both humans and laboratory animals. A 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 method allows an easy assessment of the two groups during the animals’ regeneration process, and the most striking differences between non-genotoxic and genotoxic carcinogenic-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.

TU/004 Development of an alternative testing strategy for the fish early life-stage test using an AOP network “thymoperoxidase and deiodinase inhibition leading to impaired swim bladder inflation” E. Stinckens, University of Antwerp; L. Vergauwen, University of Antwerp / ZebrafishLab Dept Veterinary Sciences; J.E. Cavallin, U.S.A. EPA / US EPA MidContinent Ecology Division; A. Schroeder, University of Minnesota-Crookston / Math Science and Technology; B.R. Blackwell, US EPA / NHEERL / Mid-Continent Ecology Division; H. Witters, VITO / Applied Bio & molecular Systems; R. Blust, University of Antwerp; G.T. Ankley, U.S.A. EPA / National Health and Environmental Effects Research Laboratory; D.C. Volz, University of California, Riverside / Environmental Sciences; D.L. Villeneuve, U.S.E. EPA / MidContinent Ecology Division; D. Knapp, University of Antwerp / ZebrafishLab Dept Veterinary Sciences 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 Fisch Embryo Acute Toxicity (FET, OECD TG 236, early development) and FELS (late development) timeframes, respectively. We defined underlying key events leading to adverse outcomes and compared their biological plausibility. swim bladder 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.
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)  
J. Cormier, MTM Research Centre Orenbro University; J. Cachot, EPOC University of Bordeaux; M. Blanch, S. Keiter, Orenbro University / MTM Research Centre  
Pesticides are used on the vineyard to protect 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 Formamidopropidimine DNA glycokalise (Fpg)) on RTL-W1 were investigated with the screening of concentrations (including the 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 (BR)). 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 sulphate/Bouillie Bordelaise 2 µg/L in RTL-W1. Similar results have been found in previous investigations using Anguilla anguilla exposed to 0.116 mg/L. Specifically for copper sulphate 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 LC50 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). This result can prove 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-Electrolytes to Assess their Skin Sensitization Potential in Chemico  
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Allergic contact dermatitis (ACD) is a widespread environmental and occupational hazard due to chemically active substances that induce sensitization through dermal contact. Typically, ACD is initiated by electrophilic compounds that are able to penetrate the stratum corneum to form antigens by reaction 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 chemooassesays such as the direct peptide reactivity assay, which can be used to discriminate between sensitizers and non-sensitizer. Besides electrophilic, another group of potentially sensitizing agents is given by pro-electrolytes that can be transformed to sensitizing electrolytes either through autoxidation or me-tabolic oxidation. Assessing the skin sensitization potential of such compounds with non-animal methods is still a challenge, because many currently used assays do not have an effective 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 chemooassay for a predictive assessment of the skin sensitization potential of organic pro-electrolytes, thus com-ple-menting existing integrated testing strategies. 1 Gerberick GF, Vassallo JD, Bailey RE, Chem. G. Mornall SW, Lopetiti 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, Schüttärmann G2009. Kinetic Glutathione Chemooassay to Quant-tify Thiol Reactivity of Organic Electrolytes – Application to Unsatuated Ketones, Acrylates, and Propiolates. Chem. Res. Toxicol. 22: 742-750. 3 Rorije E, Aldenberg T, Buis H, Kroese D, Schüttärmann G 2013. The OSIRIS weight of evidence skin sensitization tool. Toxicol. In Vitro 27: 146-156. Acknowledgement – The author thanks BMBF-funded project ProHapTox (FKZ 031A1422A and 031A1422B) 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 becoming more essential, even though their degree of contamination is 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 Lagon, Puerto Camargo and Verde Lagoon, Veracruz) and 2 beaches located in the Mexican Pacific (Papaoa 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 homogeneous of fish hepatopancreas of Pteria sterna. This test evaluates the inhibition of the 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 > Papaoa. 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.
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 simulation of the liver metabolism with liver S9 subcellular fractions. Most commonly, batch-to-batch variations are high. Therefore, these error-prone characteristics applied per assay. In addition, due to the biological origin of this resource the variability. This work has received support from the EU/EFPIA Innovative Medicines Initiative under grant agreement 122826. To confirm initial results.

TU011 CONAE1: Consortium for in vitro Eye Irritation testing strategy

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TU011

TU012 Prediction of phenol ecotoxicity using an appropriate classification into Mechanisms of toxic action.

F. Bauer, P. Bierheil, KREATIS

TU012

TU013 Predictive QSAR models to assess the toxicity of piperidinic compounds to Aedes aegypti mosquitoes

A. Douzet, Université Paris Diderot / ITODYS, E. Pansu, Université of Insularia / Department of Theoretical and Applied Sciences; W.J. Doucette, Utah State University / Utah Water Research Laboratory; G. Deviller, SOLVAY / TERA PRAFS HSE

TU013

TU014 Reducing animal products in metabolism studies - S9 subcellular fractions produced from cells in chemically-defined medium

A. Schrey, H. Xiao, EWOMIS; H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis; B. Thalmann, EWOMIS

TU014

TU015 Normalization of acute toxicity data to freely dissolved concentrations provides common basis for comparison between zebrafish and rodents

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TU015

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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/mL), providing a common basis for comparison across toxicological species. The results of the transformation of toxicity studies in rodents exposed by oral, inhalation or intravenous routes (i.e., LD50 values in mg/kg or LC50 values in mg/m³) were transformed to blood plasma concentrations. The transformed toxicity data show log-linear relationships between LC50 and the octanol/water partition coefficient (logKow) 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.

TU/016
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 cellular 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 the data. Therefore, a method validation 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, phospho- 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 (FP117535).

TU/017
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 last 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, i.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 stem cell toxicity on single cell level and on the route 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 observe significant apoptosis in stem cells via light 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.

TU/018
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 model organism and plant toxicity. Between the last two link between these. Evidence for the last mechanism might not always be unequivocally determined, particularly as the endocrine system consists of diverse endocrine axes, e.g. the hypothalumus-pituitary-gonadal (HPG) axis, the hypothalamus-pituitary-thyroidal (HPT-) axis, and the hypothalamus-pituitary-adelenal/interrenal (HPA/-1) 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 axes are very similar. In the following, 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/-1 axes, and one connection between the HPT- and HPA/-1 axes. Central KEs identified across axes were altered aromatase activity, and altered expression and function of proteins 11β-Hydroxysteroid Dehydrogenase (11β-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.

TU/019
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 vivo and in vitro. 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, testosterone, 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

TU/020
Mode of Action (MOA) assignment classifications for ecotoxicology: Overlap and consistency of available schemes
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Sustainable Consultant Toxicology and Environmental Research Consulting; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; J. Brill, The Procter & Gamble Co / Environmental Stewardship and Sustainability; D. De Zwart, DUZ Ecotoc / Centre for Sustainable Environment and Health; M.R. Embry, ILSI Health & Environment, Inc. / American Pharmacists Association; K. H. Isern, Environmental Sciences Institute (HESI) / Health and Environmental Sciences Institute HESI; M. Hodler, European Commission Joint Research Centre / DG Joint Research Centre IHP EURIL ECVAM; T. Norbert-King, US Environmental Protection Agency; H. Sanderson, Aarhus University; D.T. Chang, United States Environmental Protection Agency Agency / National Exposure Research Laboratory; P. Wilson, SANOFI 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 making reliable predictions of their risks for the purposes of risk assessment. The most commonly used MOA classification schemes for ecotoxicology include Verhaar and Ruusum (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 Ruusum includes eight classifications. MOA tox 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 MOA tox 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 structured 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’a

TI/021 Amiodarone and Tertefenide Induced Cardiototoxicity in Developing Zebrafish Embryos (Danio rerio) D. Patel, J. Rana, Jai Research Foundation / Ecotoxicology; N. Khan, Jai Research Foundation / Chemistry; C. Callol, Jai Research Foundation Drug-induced cardiac toxicity 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, 2.5, 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 a histamine H1 receptor antagonist. The 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 cardiototoxic effect when exposed to early development. The present research is based on the concept that drugs or chemicals that cardiotoxicity effect should be checked as it is valid and reliable model for rapid prediction of chemical toxicity during drug research and development.

TI/022 Examination of the relevance of Hansen Solubility Parameters to prediction of ecotoxicity and bioconcentration P.R. Fisk, Saxo House; M. Crookes, Peter Fisk Associates Ltd. 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 calculated using automation needs that 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. A fish and the toxicity of substances in single 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 reliable 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 medium. 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.

TI/023 In vitro characterization of the toxicity of selected emerging contaminants to lake trout N. Papage, RWTH Aachen; S. Beitel, University of Saskatchewan - Toxicology Centre / Department of Chemical and Environmental Engineering; B.K. Esiner, University of Saskatchewan / Department of Chemistry; H. Eckenstein, Innovative Environmental Services (IES) Ltd / Environmental Sciences Institute (HESI); H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis; M. Hecker, University of Saskatchewan / School of the Environment and Sustainability and Toxicology Centre 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 17α-ethinylestradiol (EE2), prozac, and silver nanoparticles (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 pieces and placed into a 24-well cell culture plate containing supplemented L-15 media. Tissue explants were exposed to increasing concentrations of forskolin, proclorox, EE2, Prrozac, 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 qPCR. This study is part of a larger ongoing research effort that involves investigating the potential of these and other 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.

TI/024 Challenges with Algae Growth Inhibition Test including Recovery Based on OECD Guideline 201 with Non-Standard Algae Species S. Höger, Innovative Environmental Services (IES) Ltd / Environmental Technology; H. Eckenstein, A. Dupont, J. Scheiretmüller, Innovative Environmental Services IES Ltd The 200 series of OECD Guidelines for the testing of chemicals propose various species with which the testing shall be performed. Basis for these 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 these studies highlight a highly important topic of research in order to make sure 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 rerегистration 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 preparation or the proper test design, the performance of the test itself and the interpretation of the test.

TU025
Evaluation of bioconcentration potential of several pharmaceuticals based on log DBCF 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 Kow > 4.5 to support the Persistence, Bioaccumulation and Toxicity (PBT) assessment and in Phase II, Tier A for pharmaceuticals having a log Kow > 3. The recommended protocol for bioconcentration is OECD Test Guideline 305. Bioaccumulation in Fish, Aqueous and Dietary Exposure. Based on the current OECD 305 protocol, approximately 200-300 fish per study may be required to determine a steady-state (BCFSS) and a kinetic (BCFK) 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 a result, a lower number of test animals may be used for substances in which uptake and distribution 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 Kow 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 Kow trigger and ‘B’ classification criteria, BCF < 1000 (US) and < 2000 (EU), the suitability of the current trigger was assessed using BCF and log Kow 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 P or B. In addition, the BCFv values obtained using standard OECD 305 test method were compared to the estimated kinetic (BCFKv) 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 Kow 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 (137Cs-containing particles) on luminescent 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 luminescent bacterium is a proper tool for studying the influence of 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 - abolition of effect (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 radiations. The linear dose-effect relationship 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 polyelectrolyte films (liquid and solid courses of beta-particles, respectively) showed that activation of the intracellular bioluminescence process can take place without penetration into cell membranes. So, some bacteria may have the property of detecting and discriminating 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 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 isoucosane. 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 combination of QSAR and the dominant predictive 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 assessment.

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 CO2 – supply from the surrounding air the algae cannot grow. Therefore these algae need other carbon sources, e.g. NaHCO3. 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 algae growing 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 (EC50 = around 0.7 mg potassium dichromate/L) and the open (EC50 = 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 EC50 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 protection of experimental 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 ER antagonist. 17β-estradiol and Flutamide were selected as androgen receptor (AR) agonist and antagonist and Iletrazole 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 and hatching 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 improve 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.

TU032
Chemoassay Profiling to Characterize the Skin Sensitisation 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 contact allergen bound to an organic electrophile. The potential of electrophiles to form adducts with proteins partly depends on their hydrophobicity and conjugating and oxidizing properties. Organic electrophiles include paints, 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 alternates to replace this animal test. In this context, chemoassays have been recognized as promising 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 their test compounds significantly evaporate from the murine skin, and thus are less available in vivo to react with nucleophilic skin proteins (as compared to chemoassay conditions). These volatile compounds are typically excluded from respective 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 with the concept of chemorunavailability. The latter enables one to specify 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 PreHapTox (FKZ 031A422A and 031A422B) for financial support. [1] Böhme A, Thaens D, Paschke A, Schürmann G 2009. Kinetic glutathione chemosay to quantify thiol reactivity of volatile electrophiles; 2010. Kinetic glutathione chemosay to quantify thiol reactivity of volatile electrophiles. Chem. Res. Toxicol. 23: 1420-1427. [2] Böhme A, Schürmann G 2016. Chemoavailability of organic electrophiles − Impact of hydrophobicity and reactivity on their aquatic excess toxicity. Chem. Res. Toxicol. 29: 952-962.}

TU031
Evaluation of skin sensitisation potential of 4-tet-butylphenol and 4-(benzylxoy)phenol using in vitro skin sensitisation assays
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Contact dermatitis is an inflammation of the skin characterised by redness, itching, burning and inflammation of the skin, which can be caused by 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), KeratinSen, USens, hClat have been developed and accepted for the prediction of skin sensitisation potential. p-tetary-butylphenol formaldehyde resins (PTBP-FR) are widely used as binders in industry and in numerous materials of everyday use, such as adhesives, or as a leached out component, resulting in exposure of the skin to substances in the environment. In various in vitro assays like Direct Peptide Reactivity Assay (DPRA), Amino acid Derivative Reactivity Assay (ADRA), KeratinSen, USens, hClat have been developed and accepted for the prediction of skin sensitisation potential. p-tetary-butylphenol formaldehyde resins (PTBP-FR) are widely used as binders in industry and in numerous materials of everyday use, such as adhesives, or as a leached out component, resulting in exposure of the skin to substances in the environment. The objective of the present study was to determine the sensitizing potential of 4-tet-butylphenol & 4-(Benzylxoy)phenol (constituents of PTBP-FR) on human skin in chemico and subsequent sequestration in the liver, resulting in overall marked elevation of this class of compounds is required. In this work we compared the acute aquatic toxicity data for those substances seem to follow the same relationship where this component substitution tolerable for the zebrafish metabolism should be assessed possibly interfering with nutritional requirements. Therefore, the extent of component substitution tolerable for the zebrafish metabolism should be assessed possibly interfering with nutritional requirements. Therefore, the extent of component substitution tolerable for the zebrafish metabolism should be assessed possibly interfering with nutritional requirements. Therefore, the extent of component substitution tolerable for the zebrafish metabolism should be assessed possibly interfering with nutritional requirements. Therefore, the extent of component substitution tolerable for the zebrafish metabolism should be assessed possibly interfering with nutritional requirements. 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Therefore, the extent of component substitution tolerable for 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 toxicity 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?
F. Bauer, P. Bicheler, KREATIS
Contrary 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 reactive 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 surface of a protein, they could 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 a dehydration of extracellular medium. Indeed it is expected that only non-ion, protonated form of acids could cross the membrane by passive diffusion.

TU035
SETAC Animal Alternatives Interest Group
A. Liilicnicen, NIVA Norwegian Institute for Water Research
Interplay between nutritional factors and chemical toxicity (P)
Tu036
The role of nutritional status on tissue contaminant concentrations
R. Shore, Centre for Ecology & Hydrology (NERC); M.G. Pereira, Centre for Ecology & Hydrology / Lancaster; L. Walker, E.D. Potter, Centre for Ecology & Hydrology
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 diphenyl ethers (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 renaturation or outcompartmentalization 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 translational 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 considered fish feed. Fish fed with 10% maize showed growth slower at 120 hpf (hpf). During these 5 days multiple endpoints were evaluated. Survival was scored to controls. Embryos exposed to the non-aromatizable androgen, 17β-ethinylestradiol (EE2) was chosen as ER agonist, fulvestrant (DPRA & ADRA). The mean percent depletion of cysteine and lysine for 4-tert-butyl were found to be 3 µM & 24 µM respectively. Thus, from the results of the development and need of a new marine cyanobacteria test for the 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 surface of a protein, they could 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 the dehydration of extracellular medium. Indeed it is expected that only non-ion, protonated form of acids could cross the membrane by passive diffusion.

TU038
Fatty Acid Characterisation of Nile Tilapia (Oreochromis Niloticus) in Fresh and Brackish Water
A. Abiodun-Solanke, Federal College of Fisheries and Marine Technology, Lagos / Fisheries Technology
Fish are 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-ólomi lagoon of Lagos State and Eleyere 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 content of the fatty acid 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 water 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 growth and preventing chronic diseases in humans. KEYWORDS: Fatty Acid, Nile Tilapia, Brackish and Fresh Water.
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 examines, in two different species of primary producers, changes in 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 μg/kg/day). 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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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 Scrubiculata plana have been selected because of their large tolerance of salinity variation allowing to study biological effects on the whole spectrum of salinity and the transition from marine to fresh water. The scope of this study is to assess 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 psu. The study was repeated 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.

TU041
Native (Mytilus galloprovincialis) versus non-indigenous (Xenostrobus secursis) mussels in Biscay Bay: Different sensitivity of biological responses against environmental pollution

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The Nerbioi-Bizkaibar (Biblao) 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 after-effects, such as biological invasion flourish. The Australasian mussel Xenostrobus secursis is an alien species that has consistently been reported in heavily anthropized European estuaries in the last decade. X. secursis coinhabitants estuaries with native Mytilus galloprovincialis targeted in Biomonitoring programs. Both species can be readily discriminately morphologically and anthropologically although molecular markers (i.e. G15) 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). This study was designed to understand the variability of Cd uptake, differences in Cd subcellular distribution and accumulation between non-forced exposure system has shown that this approach can provide important information about the role of the contaminants as habitat disturbers even when no possibility to avoid it. This approach, here called forced exposure, allows an ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment. The theoretical basis, the operational advantages and ecological risk assessment.
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 aviators. 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 biotic effects, determined by a gradient or even patch 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 plants results in relatively high concentrations of heavy compounds in surface waters. However, possible effects of metformin and sacrose in aquatic organisms are far from being understood. The aim of this study is therefore to investigate influences of metformin and sacrose 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 µg/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 sacrose concentrations (0, 10, 100, 1000 µg/L, 1000 µg/L, 1000 µg/L, 100 µg/L sac.). 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 heat beat rate in brown trout larvae. In both experiments, the blood sugar level was tendentially lower in metformin-exposed fish (in 1000 µg/mL metformin) compared to the control and there was no influence of metformin on the metabolism of trout muscle (muscle tissues were not available to be presented (analyses not yet finished). This work is part of the project Efl-Net (Effect Network in Water Research) funded by the Wasserwertverband Baden-Württemberg. By a multi-disciplinary approach, antibiotics, artificial sweeteners and antidepressants as well as their metabolites and transformation products are studied from the point of action of 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 freshwater ecosystems and marine ecosystems for risk assessment. The wide range of species present in marine ecosystems makes it difficult to determine which species are representative for the marine ecosystem as a whole. Therefore, 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 crustaceans is considered to be crucial for the ecosystem function, those should be placed on the list of criteria 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-dichlorovinylphosphate) on Artemia franciscana
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Dichlorvos is a highly toxic organophosphate. In the European Union (EU), 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 these issues are some of the most 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, LC50 (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 (Litopenaeus duorarum) and American lobster larve (Homarus gammarus). 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 Endosulfan in the Yangtze River Delta Region was conducted. Endosulfan is a pesticide which has been widely concerned due to its high toxicity and persistance, 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 Linnaceae. 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 µg/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.
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 marine invertebrate community. 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 are determinant to the fate of BT residues along the studied area. The ingestion of only about 100 g of “locate” foot tissue (4 to 8 organisms) 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 that the local human health risk associated to food consumption, regulatory actions toward environmental protection and food safety of local populations should be implemented.

TU051 Assessment of sperm quality in palaeonid 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 Palaeonidae serratus to use it as a marker of sperm quality. Indeed, due to the characteristics of their spermatozoa, the measurement of DNA integrity is one 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 spermatozozoa toward a H₂O₂ 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 Palaeonidae species. Finally, the sensitivity of P. serratus spermatozoa was assessed after in vitro exposures toward model genotoxics displaying various modes of interaction with DNA (i.e. UV-C -5 30 sec; H₂O₂; 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 µL L⁻¹; carbamazepine, 0.1-10 µL 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 when the dose was increased in all cases; however, 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 Palaeonid prawns in genotoxicogenetics. In conclusion, the previously underlined potential of the Comet assay as a marker to assess the contamination impact on the sperm quality in Palaeonidae 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 EC50 of 1000, 2200 and 2342 mg/kg respectively. Similarity, the *T. battagliai* chronic test showed a survival EC50 of 396.2 mg/L with a NOEC and 78.4 mg/L respectively. All these results and their subsequent 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 EC50 of 214 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 EC50) 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 predict 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-butanon (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 toxicity and developmental toxicity tests 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 212). The toxicity testing revealed a very low acute and development toxicity for MEK and 2-MTHF compared to 2-MF and 1-Octanol. For MEK and 2-MTHF, EC50>1000 mg/L were found for *D. magna* immobilization and LC50>2000 mg/L (2-MTHF) and >4000 mg/L (MEK) were found for *D. rerio* embryo toxicity, EC50<35 mg/L were determined for acute immobilization induced by 2-MF and 1-Oct and fish embryo toxicity testing revealed lower LC50 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 > MEK > 2-MTHF. These findings in the 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”


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 (FELS) OECD 203 test with an AOP-derived 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 AOP based on a model initiating 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 TCBA is no longer the highest positive DIO1 agonist, while another compound became the most 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 Lemma 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, *Lemma gibba*, *Lemma minor* and *Lemma pauciaustia* as test organisms. The sensitivity of several ecologically relevant parameters (increase in front area, root length after regrowth, maximum and effective quantum yield of PSII and maximum electron transport rate (ETR50)) were compared after 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 *Lemma* assays. Sensitivity ranking of endpoints, based on EC50 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 ETR50 (4.7 – 10.3 µL g-1) for paraquat and effective quantum yield (6.8 – 10.4 µL g-1) for diuron. Growth and chlorophyll a fluorescence parameters in all three *Lemma* species were sensitive enough to detect toxic levels of diuron and paraquat in water samples. Biomass parameters of all herbicides were in line with our AOP and illustrate how AOP-derived information can be used for comparing the sensitivity of all tested compounds. The work conducted here was a 5 day semi static assay. For the chronic tests, as well as their semen. In first time, the methods to collect and to maintain spermatozoa were developed and adapt some of these tests in miniaturized fluidic systems in order to perform experiments in a reduced volume and thus accelerate the data acquisition phases. Preliminary tests have been performed to follow the growth of *Pseudokirchneriella subcapitata* in microdroplets and thus accelerate the data acquisition phases. Preliminary tests have been recorded during the maturation. No other effect in term of cytological and生化影響 by chronic tests have been noted. 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.

**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 inhibitory effects of *Daphnia magna* (in vitro) on key enteric bacteria, *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 bacterial concentrations. Maximum feeding rates were >10^4 bacteria h^-1, clearance rates were 1-6 ml h^-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 NH3-N. The lowest observed effect concentration (LOEC) forunionized ammonia was 0.09 mg L^-1 NH3-N. In contrast, filtering of *E. coli* by *D. magna* was not inhibited 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 key 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 in wild milkfish (Chanos) from the aquaculture farms in southern Tanzania (Mtwara) and Zanzibar islands was 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 liver 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 on fish 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'-DDE was detected in milkfish from Jozani and Shaktani ponds in levels of 14 and 3.5 ng/g lw respectively, while 4 ng/g lw in wild mullet from Pemba. Levels of 2,4,6-Trichloroanisole were low and ranging between < LOD-5.6 ng/g lw. The highest levels of 10 ng/g lw were found in milkfish from Shaktani pond at Unguja (5.65 ng/g lw). The PCB pattern was dominated by PCB 153>PCB 180>138. Levels of HCB, HCHs, and non-trans-norleucine were low and may reflect background levels. PBDEs were detected in milkfish from Mtwara and Mullet from Pemba, the highest levels of 0.13 ng/g lw in the fish from Shaktani 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 worldwide. 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 organism 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 (EC50): Daphnia acute immobilization test determines the concentrations which 50% of the daphnia are immobilized (EC50); and Acute fish toxicity determines the concentration which is lethal to 50% of the fish (LC50). 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 disturb 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 Gammarus, 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 (EE2) and the cyproterone acetate (CPA), EDCs commonly studied in vertebrates and the estrogenic hormone VT on the spermatogenesis of the freshwater amphipod Gammarus pulex males, by assessing the number of spermatogonia produced as well as the their length. Results in control conditions (i.e. unexposed males) revealed that the presence of Dizycocela duebenum (the only one microsporidia VT in the studied Gammarus pulex population) significantly reduces the production of spermatogonia compared to unparasitized males, without having an effect on their length. In addition, it appeared the EE2 did not affected neither the number of spermatogonia produced, nor their length, while CPA stress caused a decrease of the spermatogonia production in parasitized G. pulex males compared to respective controls. This work highlighted the potential of both endocrine disrupting vertebreto-invertebrate interactions from the cross-talk of microsporidia with the endocrine system of the host. In addition, the presence of microsporidia VT appeared to be a confounding factor which could lead 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 coastal reference sites in Sweden since 1988. The selected reference sites are characterised by no or small local sources of contaminants. The fish health is a result of early detection of sub-lethal effects caused by chemicals. In addition, the fish are potential indicators of ecosystem 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 coastal 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, the samples of perch liver were selected for RNA sequencing. The main 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 transcriptome that can be used in future studies.

TU061 Effects of imidacloprid on the escape and agonistic behaviors of crayfish
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For the release of crayfish 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 immediately after the exposure they were tested for escape responses. Escape responses were characterized using three behavioral tests: a vertical escape test, a horizontal escape test, and a rearing test. 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 = tilt 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 experimental groups responded with the same frequency to the stimulus in the 100 µL/g group an increase in the frequency of neutral responses was detected already after 4 days exposure and after 8 days in the low (1 µg/g) exposure group. Several studies have reported levels of imidacloprid exceeding this study’s lowest exposure scenario, 1 µg/g, and we therefore conclude that the 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 cholinergic 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][BF4]), 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][BF4], on mussel hemocytes, as well as whether acetone could mediate their toxic potention. In addition, since understanding ILs’ mode of action with and/or without their presence of related solvents, toward non-target species is of great concern for their development, application and safety, guaniidine hydrochloride (Gnd HCl), a chautropic agent and strong macromolecules’ denaturant, was used for elucidating the hydrogen bonding network among [omim][BF4], water and/or [omim][BF4]/water/acetone with exposed cells and shed light to [omim][BF4] 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. Therefore, 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][BF4] ranging from 0.2 to 1 mg L-1 were able to induce cytotoxic (<50% reduction of cell viability), oxidative (increased levels of O2- 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 1 mM showed a significant diminishment of [omim][BF4]-mediated toxic potency in all cases. To our knowledge, the current study showed for the first time that chautropic 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][BF4] 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 selenium) 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 food web. 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. In order to 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 levels. The project will be conducted in collaboration with 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 days amipdops (Hyalella azteca) were allowed to graze on the exposed biofilms, followed by analysis of the Se concentration in the amipdops to determine the trophic 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. Conclusively, this will be the first study to explore the effects on development of P. promelas embryos injected with SeMet to gain insights into early life stage sensitivity and mechanisms of Se toxicity. The 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 (Benzenophene-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 ecosystems inhabiting coastal areas, notably the affected coastal ecosystems 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 classes. In this study we aimed to evaluate the effect of short exposures of BP-3 on Zoanthus sp. photochemical performance and zoanthallene density. Using a non-invasive method and replicated mini-colonies of 4 to 6 polips, the maximum quantum yield of photosystem II (Fv/Fm) 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 zoanthallene 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 zoanthallene 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 coastal ecosystems. Key-words: corals; emerging contaminants UV filters; Benzophenone-3; marine ecotoxicology
LABORATORY ASSAYS WITH NON-FORCED EXPOSURE TO PREDICT THE PREFERENTIAL SPATIAL DISTRIBUTION OF FISH IN TWO ECUADORIAN RIVERS

C. Araujo, Consejo Superior de Investigaciones Científicas / Department Ecology and Coastal Management; V. Vera-Vera, Universidad Laica Eloy Alfaro de Manabí / Coastal Department of Research; D. Saldivar, Universidad Eloy Alfaro de Manabí / Departamento Central de Investigación; B.N. Ariza, D. Griffith, S. Erazo, Universidad Laica Eloy Alfaro de Manabí (ULEAM) / Central Department of Research, P.V. Jentisch, Escuela Politécnica Nacional / Department of Chemical Engineering; R. Jaramillo, L.A. Ramos, Ecuadorian Agency for Quality Assurance in Agriculture / Agroalidad; J. Blanco, Inst. Ciencias Marinas de Andalucía / ECOLOGY AND COASTAL MANAGEMENT; M. Moreira dos Santos, CFE - Centre for Functional Ecology / Centro de Ecología Funcional; R. Ribeiro, Universidade de Coimbra / Life Sciences Laboratory. Laboratory assays to predict the possible effects on microbial communities, the main respirers and recyclers of the large pool of dissolved organic matter, remain uncharacterized. The objective 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 exposure and control treatments and showed 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

C.H. Soares, Universidade Federal de Santa Catarina / Biochemistry Department; K. Brauco, Universidade Federal de Santa Catarina; M. Barbosa Xavier, Universidade Federal de Santa Catarina / Biochemistry; W. Weis, J. Baptista, Universidade Federal de Santa Catarina; I.C. Cord, Federal University of Santa Catarina / Biochemistry. 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 Iacorubí River Basin. The river system is connected with a large estuary and a river that flow to the ocean. We challenged natural polar communities with mixtures of OPs (aromatic and aliphatic hydrocarbons, chlorophenols, and sterols) in water and sediment samples. For the purpose, three sites with two from the basin are analyzed for each river. Results show that the combined use of OPs is necessary to provide data for ecological risk assessment of OPs in sediments. For this purpose, the OP concentrations were normalized for the soluble Ag dose. After this normalization, tissue distribution and Ag concentrations were measured by ICP-MS. After treatment by AgNPs, there was a low absorption of Ag, the absorption after treatment with NM-300K was estimated by ICP-MS. The results show the non-availability of Ag after treatment with AgNPs. After treatment with AgNPs, the tissue distribution of orally administered AgNPs was compared with the tissue distribution of orally administered Ag salt. The results show that the combined use of AgNPs is necessary to provide data for ecological risk assessment of OPs in sediments.
lessons learnt from human models (P)

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 ubiquitous in our everyday lives as they replace 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 nonexistent to properly assess this emerging concern. Here, we evaluated the lung toxicity of 3 leading LIB particles (LiFePO4 or LFP, LiTiO2 or LTO, and LiCoO2 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. C57/BL/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 after 18 h and 3 days after exposure, suggesting different mechanisms of action. Persistent inflammation was observed 2 months 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 months after exposure by scanning electron microscopy/energy dispersive X-ray spectrometry (SEM/EDX). 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) sulphate affects dendritic cell maturation

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The medical application of gold nanoparticles (GNPs) is promising due to their high biocompatibility and tunable properties. However, insufficient knowledge about their interactions with biological systems and complex 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) by scanning confocal laser microscopy. Characterization data indicated that nickel(II) ions did not adsorb onto the GNP surface, but caused 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 nanoparticles’ 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 metalized 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 diameter 3 nm. The rats were treated either by oral gavage or by IV injection. During a 24-hour observation period after IV injections, 24-hour urine samples were collected. Total Ag concentration was measured by ICP-MS. After 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 liver, spleen and pancreas, and 6% 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 AgNO3. 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, however, showed a significantly higher concretion of 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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TiO2 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 TiO2 NPs and PCB77 influence each other’s uptake, fate and toxicity at different levels of biological organization in the model organism rainbow trout (Oncorhyncus mykiss). The uptake, fate and effects of TiO2 NPs and PCB77 at the cellular level was investigated by exposing cultures of the rainbow trout liver cell line RST-W1 to the substances both alone and together. Transmission electron microscopy analysis demonstrated that TiO2 NPs were taken up via chlatin-independent endocytosis and routed to multimellar vesicles. Intracellular TiO2 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 not significantly contaminated by TiO2 NP-induced oxidative stress. Yet, higher agglomerate burden of TiO2 NP affected the cells’ response to subsequent PCB77 exposure (0.1, 1 and 10 µM). Thus, NP-containing cells showed higher cytochrome P450 P450 1A (CYP1A) expression levels and alterations in their antioxidant response. In order to investigate if combination effects of TiO2 NP and PCB77 occur as well at the organism level juvenile rainbow trout were exposed via the diet to TiO2 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 TiO2 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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reagents has been tested as well. Some differences in cellular responses to the role of mitochondria entering the cell, ongoing research will investigate the possible internalization and pronounced only at the highest exposure concentration. In general, both cell lines (NMs), their possible adverse effects on biological systems need to be identified. This study was supported by the European Union H2020 Program under grant agreement no. 696656-Graphene Flagship Core1.

T1/076 Evaluation of the toxicity of graphene-related nanomaterials on fish cell lines J. Kalmus, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria / Environment; C. Merino, Grupo Antolin Ingenieria SA; M. Fernandez-Cruz, J. Navas, INIA - National Institute for Agricultural and Food Research and Technology / Environment

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 and concentration were kept constant. A variety of in vivo toxicity assays were used, including a specific assay to accurately assess Ag NPs toxicity in primary cultures of E. fetida coelomocytes. These combined techniques were important to regard correctly the experimental reproducibility and to ensure that the responsiveness of cells against toxicants may affect bioavailability and hazard. In this work, the relationship between different physicochemical properties of NMs and increased due to lack of knowledge on their use, fate and effects, but also the lack of environmental conditions may affect bioavailability and hazard. In this study, we analyzed the impact of Cu0<sup>+</sup> on the viability of E. albidus mussel coelomocytes and Ag NPs toxicity in primary cultures of E. fetida coelomocytes. This study was supported by the EVOS Horizon 2020 research and innovation programme (NanoReg2, Grant Agreement n° 646221).

T1/077 Assessment of few layer graphene and graphene oxide effects at the skin level: the role of mitochondria M. Pelin, L. Fusco, University of Trieste; V. Leon Castellanos, University of Castilla La Mancha / Department of Quimica; C. Jimenez, University of Castilla La Mancha; E. Vazquez Fernandez-Pacheco, University of Castilla La Mancha / Department of Quimica; S. Sosa, A. Tabaro, University of Trieste; M. Prato, University of Trieste / Department of Chemistry

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 goyol oxide of FLG) to native human 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 (EC50 = 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 GBNs. Further studies will be needed 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.

T1/078 Tissue specific differences of copper oxide nanoparticle toxicity in the marine mussel Mytilus edulis a. champi, Universitat Catholique de Louvain; C. Lavevre, MMS UCO; A. Barrick, M. Bruneau, Universitat Catholique de Louvain / LUNAM Université Mer Molecules Santé MMS; C. Momeyer, Université Catholique de Louvain / UNAM Université Nantes Angers Le Mans Mer Molecules Santé MMS

The current and foresight 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 Cu0<sup>+</sup> on GST, SOD, MT and Actin mRNA genes expressions indicative that CuO NP was more cytotoxic than soluble Cu0<sup>+</sup> from the nanoparticle form. The overall results allowed to potentially novel molecular targets of CuONP and Cu0<sup>+</sup> exposures in the mussel. This work 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.

T1/079 Reproductive toxicity via JAK/STAT and TGF-β pathways crosstalk as potential adverse pathway of UV activated TiO2NPs in the nematode C. elegans: Integrated transcriptomics and metabolomics analyses L. de Jesus Kim, University of Seoul / School of Environmental Engineering; N. Chatterjee, University of Seoul / Environmental Engineering; C.P. Roca, University of Aarhus / Department of Bioscience; S. Kim, Pusan National University / Department of Chemistry; J. Choi, University of Seoul / School of Environmental Engineering

Titanium dioxide nanoparticles (TiO2NPs) 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 TiO2NPs was investigated on the nematode Caenorhabditis elegans with/without UV activation (i.e. Control, TiO2-NPs, UV, UV+TiO2-NPs). Toxic Mechanism study was conducted using integrated transcriptomics and metabolomics under the exposure condition that caused reproducible 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 TiO2-NPs led significant reproductive and lethal toxicity through oxidative stress to C. elegans. DFG analysis and pathway enrichment analysis were performed to identify pathways significantly expressed by TiO2-NPs alone, whereas, 3,625 genes by UV alone and 3,286 by UV.
activated TiO₂NPs exposure. Pathway analysis suggested possible involvement of JAK/STAT and TGF-β pathways in toxicity of TiO₂NPs, which was further validated by observation of increased gene expression of those pathways. Comparative analysis on C. elegans response across UV activation and TiO₂NPs exposure using loss-of-function mutants of genes in these pathway revealed that JAK/STAT and TGF-β pathways were functionally specific in C. elegans. The outcome of our study will contribute significantly to better understand the mechanism of photocytotoxicity of TiO₂NPs, 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. Keywords: Caenorhabditis elegans, TiO₂NPs, Phototoxication, Multi-OMICs, JAK/STAT and TGF-β 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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Nano-toxicity 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 cell effective toxicology models. However, 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 E. fetida 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 AgNO₃ (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 absorption spectrum were characterized 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 LC₅₀ 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 (LC₅₀ 36.68 and 30.48 mg/l, respectively), followed by AgNO₃ exposure (LC₅₀ 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 AgNO₃ 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 microalgae 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, 90 μg/l, for Ag NMs, and 0.85, 1.7, 3.42 and 6.83 mg/l, for MWCNTs). The highest concentration corresponded to the obtained EC₅₀ value in the standard tests. NMs were dispersed using bath or probe sonication. The ROS production was measured by using the cell permeable probes 2',7'dichlorodihydrofluorescein diacetate (H₂DCFDA) at different time points (2, 6, 24, 48, and 72h), Cellular esterases hydrolyze the probe to the non fluorescent 2',7'dichlorodihydrofluorescein (H₂DCF). In the presence of ROS, H₂DCF 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 NPs dispersed using bath sonication. Different treatment protocols, such as 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
T.F. Fernandes, Heriot-Watt University / Institute of Life and Earth Sciences

The interest in the environmental effects of emerging contaminants continues to increase due to lack of knowledge on their use, fate and effects, and 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 ENMs effects in biological systems; differentiate cells/tissue injuries due to nano and non-nanomaterials 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 taxon 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 work investigated the effects of biochar on the toxicity of AgNPs to the species Enchytraeidae 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 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 day LC₅₀ 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 Cadocera quantified by liquid chromatography coupled in a hybrid quadrupole-Orbitrap Mass Spectrometry
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Fullerenes are carbon-based nanomaterials with a particular interest in biomedical research due to their unique physico-chemical properties which include water solubility, and high stability in various aqueous matrices. Current studies on the effects of fullerene C₆₀ on aquatic organisms are not sufficient to recommend a safe concentration. In this study, we tested the effects of C₆₀ on the sub-lethal toxicity to the freshwater cladoceran C. dubia using a combination of cell viability tests and biomarkers (transcriptomics and metabolomics) and pathway analysis (transcriptomics and metabolomics) and pathway analysis. The main anthropogenic source of fullerene is hydrogenation-combustion, present on industry and combustion process. In this study we observed sub-lethal effects of fullerene C₆₀ on daphnids and compared the exposure concentrations with the real concentrations quantified by analytical techniques because the unique physico-chemistry of C₆₀ 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 C60, 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 postery till the 12th day after birthday. Endpoint as female’s fecundity, hooting frequency and their mortality. Firstly, the 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 high improved selectivity compared to traditional 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 LCn 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.

TU008

The use of proteome profiling to detect potential biomarkers for monitoring the immunotoxicity of engineered nanoparticles

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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). Analysis of the data also screened for the effect of the nanoparticles on cell viability 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.

TU006

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 material in various products. We studied PbO NPs with whole body inhalation chambers for up to 72 hours in acute experiment (4.05x10³ nanoparticles/cm³) and for up to 11 weeks in chronic experiment (3.83x10³ nanoparticles/cm³ in lower and 1.93x10⁴ nanoparticles/cm³ 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. P305/11/2315 and P303/12/4147 and by project LQ1214 funded by the National Sustainability Programme of the Czech Republic.

Agricultural and Terrestrial Plant State-of-the-art Research Linking Ecotoxicology and Exposure of Chemicals (P)

TU007

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 (NTPs). 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 NTPs differ between in-field and off-field areas. They identified some areas for further analysis and analysis that would reduce uncertainty in the risk assessment. In particular, the protection of standard species for wild species and the protection 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 NTPs was considered. Comparative analysis of 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. ErC50, ER3 or ER50). 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 NTPs (ERm for juvenile plants) to ERp for reproductive effects on mature plants, the difference in endpoints ranged between 6.25x and 8.68x. At the second workshop a framework for higher tier risk assessment for NTPs was also discussed. Furthermore it was noted that many endpoints described as a MagPie are appropriate for NTPs 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.

TU008

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 (NTP) need to be assessed. Routine testing with NTPs is done in highly standardized experiments in all studies and often with 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 NTPs. 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 in all the three locations 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 NTPs.

TU089
Phytotoxicity Assessment of Methanol and Toluene Introduced into Paddy Field by Chemical Spill on Rice (Oryza sativa) M. Park, Korea Univ. / Department of Environmental Science and Ecological Engineering; J. Kim, W. Hwang, H. Bang, K. Kim, S. Hyun, Korea University / Department of Environmental Science and Ecological Engineering

In Korea, there are many soil contamination cases by methanol (CH₃OH) and/or toluene (CH₃C₆H₄) by chemical incidents. Methanol and toluene can be introduced into soil via leached residual by underground water 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 phytotoxicity 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 EC₅₀) on the basis of the measured endpoint were calculated using SAS 9.2 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 D. Kim, J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Health Science

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 a series of processes. Therefore, data for toxicity of bisphenol A on plants were reported. This study 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-EC₂₀ and NOEC were estimated and the 21d-EC₁₀ 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 phytotoxicity 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 S.L. Nason, University of Wisconsin - Madison / Environmental Chemistry and Technology Program; E.L. Miller, University of Wisconsin - Madison / Molecular and Environmental Toxicology Center; K. Karthikeyan, University of Wisconsin - Madison / Biological Systems Engineering; J.A. Pedersen, University of Wisconsin - Madison / Soil Science

Irrigation with treated wastewater and soil amendment with biosolids are 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 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⁻, HCO₃⁻, 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 bioavailability of other contaminants. We tested the effects of different nutrient solutions on wheat plant rhizosphere conditions and uptake of lamotrigine, an anti-epileptic drug (pKa = 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 is the bioavailability and assessment of the different EC₅₀s will be discussed.

TU092
De novo transcriptome sequencing and gene expression profiling of Phoenix dactylifera L. under cadmium stress F. VANDENBULCKE, LGCe, Université de Liège 1 / LGCe EA Univ Lille Fonctionnement des Écosystèmes Terrestres Anthropisés Cité Scientifique SN Rdc F Villeneuve dAsq France; Z. Chaidaune, University of Sfax / High Institute of Applied Biology of Medicine; A. Elieuch, Faculty of sciences of Sfax University of Sfax / Laboratory of plant biotechnology

The accumulation of transcriptional changes in plants can help plants survive and resist to abiotic stress. 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, 37,684,583 clean reads were produced. The transcriptome assembly. The draft transcriptome assembly consists of 6779 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 macroporhage proteins (PdLnnrops), ATPhase family and PdMATE efflux family in addition to Cd chelator genes like phytochelatin synthase (PdPCs) and metallothionein (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 transcription accumulation of PdLCS, PdATPase, PdMATE and PdPPDR has been shown as up-regulated PdMT and PdABC expression levels. Increasing metal time exposure further induced genes expression under 0.02 and 0.2 mCd. However, a significantly important decrease of the level of expression was observed at the end of the treatment, PdMT, PdLNN and PdATPase. The P. dactylifera transcriptome presents a large set of 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 S. Duquesne, UBA, Federal Environment agency; G. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment; L. Hönnemann, S. Matecki, M. Sólk, German Federal Environment Agency UBA; R. van Wijngaarden, Wageningen Environmental Research Alterra

The aquatic environmental risk assessment (AA-PEP) 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 Lemma sp. are required in the aquatic first tier of the risk assessment scheme. If Lemma sp. is not sensitive, additional tests for other aquatic macrophytes should be performed before 2015. A major endpoint among ErCrO, ErCrO and ErCrO (growth rate, area under the curve, yield) could be used. Since 2015, the aquatic Guidance Document (AGD) (EFSJ Journal 2013; 11(7) 3290) recommends to use preferably the ErCrO. Since ErCrO and ErCrO values are often lower than ErCrO, 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 ErCrO (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 SETAC Europe 27th Annual Meeting Abstract Book 195
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 amidosulfuron. The exposure to amidosulfuron significantly reduced the growth of macrophyte, 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 recovery phase the final biomass of the exposed plants was below their initial values.

TU/095 A proposed ring-test protocol for the emergent macrophyte, Glyceria maxima, in a water-sediment system J. Davies, Syngenta / Environmental Safety; G. Arts, Alterra Wageningen University and Research Centre / Environmental Risk Assessment; K. Kuhl, Bayer CropScience AG; I. Kubitza, BASF SE; M. Ratte, ToxRat Solutions GmbH 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 workshop 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 workshop 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.

TU/096 Suitability of several dicotyledonous macrophytes as additional test species for the risk assessment refinement S. Mohr, German Environment Agency / IVV; B. Alschler, I. Janturh, German Environment Agency; R. Schmiedecke, M. Feibicke, R. Gersg, Umweltbundesamt 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 selected of macrophytes and for algae should be tested for a SSD. 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. Therefore it would be appropriate to test species of mono- as well as 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 transferred 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.

Intraspecific variation in the sensitivity of two macrophyte species to copper contamination E. Roubeau, EcoLab CNRS / Integrative ecotoxicology; S. Lorber, INRA Institut National de la Recherche Agronomique / Toxalim UMR1331; A. Elger, EcoLab CNRS / EcoLab 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). All 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.

TU/098 A proposal of novel ecotoxicity test method using automatic analysis of color change in the green alga Ulva pertusa Kjellman (Chlorophyta) H. Lee, K. Yang, M. Kim, Incheon National University / Department of Marine Sciences; T. Han, Incheon National University / Marine Science 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 eluters 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, covenlopings were placed on the bowls containing petri dishes to ascertain whether spores were released since ambient ammonia exists in the color between fully emptied thallus after spore release and dead thalli. The sporulation percentage was then calculated by the stained area over the whole thallus disk area. EC50 values obtained from the original and newly developed method were similar: 0.1166 vs 0.1457 mg L-1 for Cu, 0.0368 and 0.0629 mg L-1 for diuron, 0.1955 and 0.2047 mg 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.

TU/099 SETAC Plants Interest Group S. Loutsetti, 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 J. Sergent, SOLVAY / HSE; E. Salinas, BASF SE / Experimental Ecotoxicology; A. ECETOC TF, ECETOC 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 proposed 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 standard ecological risk assessment procedures may be inappropriate to 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, and copper nanoparticles) and for aquatic higher organisms, with the special focus on physico-chemical 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

Testing 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 aquatic 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 experimental setup is based on performing range-finding tests, and the effect of each concentration of ZnO and SnO2 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 means of EC50, EC10, and EC90 were calculated. The sensitivity of Chlorella species to ZnO nanoparticles was much more than Scenedesmus species: 72h EC50 value was 0.01 mg/l for Chlorella and 0.09 mg/l for Scenedesmus. But, Scenedesmus species was more sensitive to SnO2 nanoparticle than Chlorella: 72h EC50 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 1-3. That may pose a potential risk to aquatic environment, exerting toxic effects at the level of cells, tissues or the whole organisms. To represent potential effects of nanoparticles 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 phytoxicity area, the aquatic plants response to metal oxide nanoparticles is still poorly known. The present study evaluate the toxicological effects of titanium dioxide (TiO2), magnetite (Fe3O4) and cerium oxide (CeO2) on Spirodela polyrhiza by exploring concentration-dependent effect and changes induced at 24, 48 and 72h of exposure. Seed germination efficiency and fronds morphology were used as endpoints to assess the phytoxicity for NP concentration from 1.25 to 1000 mg L-1. The particle size and the ζ-potential of NPs in the culture solutions were measured to identify possible differences in the profile and the observed toxicity on duckweed. The obtained results reveal toxic effects of TiO2 and Fe3O4 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 Co3O4 did not produce any significant negative effect. References [1] Wick P.A., Janghorbani A.K., O'Malley E., Herrmann J., Escher B.J. 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. 286-93. [2]Sahle-Demessie E., Changsuek H., Amy Z., Bill H., Heidi G. 2016. Interaction of engineered nanoparticles with hydrophobic organic pollutants. Nanotechnology 27:284002. [3]Guo Q, Heta H, Wang J, Lu J. 2015. The properties of nickel oxide bulk and nanoparticles on the aquatic plant Lemma gibba L. Biomed Res Int. 501326. Acknowledgement- This work has been financed by the FP7-ERA-Net Susfos, 2014/00153/001 No. 29176, the Spanish Ministry of Economy and Competitiveness, CTM2013-45775-C2-1-R (MINECO/FEDER EU) and the Comunidad de Madrid Network 52013/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, two different test species with different organic and inorganic chemicals on zebrafish embryos was assessed. The simultaneous presence of C60 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 C60. 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 (AgNO3, CuSO4) on gut health in juvenile rainbow trout (Oncorhyncus 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 Pocillia 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 INOPs (maghemite) coated with citrate and perform its characterization in terms of individual shape, crystallite size, hydrodynamic diameter and zeta potential in order to grow and filter water, as well as to investigate the bioaccumulation and genotoxic effects in the guppy P. reticulata. INOPs were produced by aqueous precipitation and characterized using Transmission Electron Microscopy (TEM), Dynamic Light Scattering (DLS) and Electrophotoretic Light Scattering (ELS). Fishes were exposed to magnetic elements 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 sister chromatid exchange (SCE) frequencies. TEM results demonstrate crystalline and rounded INOP with an average size of 3.97 ± 0.85 nm. The DLS and ELS analysis showed that the INOPs has low hydrodynamic diameter and high surface charge in ultrapure water (14.1 ± 0.2 mm; -51.1 ± 7 mV) compared to reconstituted water (21.4 ± 0.39 mm; -19.5 ± 6.5 mV), indicating that the ionized citrate groups confer negative surface charge of INOPs in both aqueous medium, confirming the tendency of these NPs to aggregate/agglomerate due to low repulsion forces. P. reticulata uptake and accumulated the INOPs in an exposure time and organ dependent patterns. Results showed that the INOP 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. TEM images showed that INOPs 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. Fine tailings slurry fraction a mixture of small particles < 100 μm and may 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 and to measure and L/E/IC50 values calculated using nonlinear regression models. These tests (with some adaptions). Lethal and sublethal endpoints were used. Values of 787±6.2 mg/L for most species, including also

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, polyethylene glycol, poly (vinylpyrrolidone), streptavadin, 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 and so deleterious effects of growth inhibition and cell viability loss in the food chain. We have used two species: Anabaena sp (cyanobacterium) and Chlamydomonas reinhardtii (microalgae). 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, the 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 CBP4337 [3]. Different parameters have been measured in Chlamydomonas reinhardtii like growth inhibition, chlorophyll fluorescence, membrane damage, 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, Crobiie-Staunton K, Sales G, del Puerto-Morales M and Volkov Y. IEEE TRANSACTIONS ON MAGNETICS, 2013:49 :1 Correa-Domingues-Pitães I, Bolte C, Falco E, Santiago S and Rosal R. Toxicological sciences. 2011 119(1), p. 135–145. Acknowledgements: This research is supported by CTM2013-45775-C2-2-R and CTM2013-45775-C2-2-R grants from MINECO/FEDER. JHG is working under FPI contract (MINECO/FEDER).

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) and its major components (LDH) for active corrosion protection of metallic structures of the environment. Several sub-lethal and lethal endpoints were measured and L/E/IC50 values calculated using nonlinear regression models. These results indicated that the novel antifouling nanomaterial SiNC-DCOIT can be a promising solution with high antifouling activity. In conclusion, SiNC-DCOIT can be a promising solution with high antifouling activity.

TU111
Hazard assessment of an antifouling biocide encapsulated in silica mesoporous nanocapsules (SiNC-DCOIT-Ag)
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Silica mesoporous nanocapsules (SiNC) with antifouling properties and low persistence in the environment, such as DCOIT (2,3-dichloro-5,6-dicyanobenzoic acid), have been developed in the last years. Booster biocides to increase the hazard potential of the NM. Despite of higher bioaccumulation of triclosan and benzophenone-3 in the present study aims to assess the hazard of this novel anticorrosion nanomaterial (LDH–MTB) and its major components (LDH–NO₂–MTB) 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–NO₂) and LDH–MTB were carried out using bacteria (Vibrio fischeri), cyanobacteria (Arthrospira maxima), microalgae (Isochrysis galbana, Nanochloropsis gaditana, Phaeodactylum tricornutum, Tetraselmis chui), rotifers (Brachionus plicatilis), bivalves (Cerastoderma edule, Mytilus edulis, Ruditapes philippinarum), polychaetes (Hediste diversicolor), crustaceans (Acartia tonsa, Artemia salina, Palaeoniscus varians), and echinoderms (Echinococcus lividus). The exposure concentrations ranged between 0.10 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/IC50 values calculated using nonlinear regression models. These results indicated that the novel nanomaterial SiNC-DCOIT-Ag as a promising antifouling coating with low environmental hazard and risk.

TU109
Hazard assessment of innovative anti-fouling nano-based solutions to marine species
J. Figueiredo, University of Aveiro / Biology; R. Martins, Department of Biology, University of Aveiro / Department of Biology and CESÂM; T. Oliveira, Smallmatek, Small Materials and Technologies Lda.; S. Ribeiro, University of Aveiro / Biology; F. Maia, Smallmatek - Small Materials and Technologies, Lda.;
A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM, 3810-193 Aveiro; J. Tedim, Universidade de Aveiro / Department of Materials and Ceramic Engineering CICECO; S. Loureiro, Universidade de Aveiro / Biology 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, biofouling agents 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 (Arar et al. 2009). As a consequence, a new generation of biocides were developed with low toxicity and the ability to control the environment 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 whole 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 AgNo3). Ecotoxicity tests were carried out with eleven marine species, including bacteria (Vibrio fischeri), microalgae (Isochrysis galbana, Nanochloropsis gaditana, Phaeodactylum tricornutum), rotifers (Brachionus plicatilis), bivalves (Cerastoderma edule, Mytilus edulis), polychaetes (Hediste diversicolor), crustaceans (Artemia salina, Palaconvenus litoridus), and echinoderms (Paracentrotus lividus), following standard tests (with some adaptations). Lethal and sublethal endpoints were used. Values of LC50 for SiNC-DCOIT-Ag (0.014–5.414 mL/L) were higher than the estimated values for both the early DCOIT-Ag (0.014–0.1 mL/L) and the late DCOIT-Ag (0.014–0.1 mL/L) 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

R. Martins, Department of Biology, University of Aveiro / Department of Biology and CESAM; J. Figueiredo, University of Aveiro / Biology; T. Oliveira, Smallmatek, Small Materials and Technologies Ltda.; S. Ribeiro, University of Aveiro / Biology; A.M. Soares, Universidade de Aveiro / Department of Biology & CESAM, 3810-193 Aveiro; J. Tedim, Universidade de Aveiro / Department of Materials and Ceramic Engineering CICECO; S. Loureiro, Universidade de Aveiro / Biology; F. Maia, Smallmatek - Small Materials and Technologies, Lda. 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 CO2 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®), are widely used in state-of-art protective coatings of metallic submerged structures. Recently, the encapsulation of biocides in silica nanocapsules (e.g., silica-coated antimicrobial 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 efficiency 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, rotifiers, bivalves, polychaetes, crustaceans and echinoderms, following standard tests (with adaptations). Several lethal and sub-lethal parameters were used for the hazard assessment. The non to antifouling nanomaterial was always less toxic than the biocide (L/E/IC50=0.016–38.20 mL/L and 0.012–1.956 mL/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 efficiency 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 synthetic nanomaterials (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 TiO2 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 glutathione peroxidase, S-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 TiO2 in form of TiO2 NPs and the other one was 100 ppb of TiO2 and AgNO3. The other two treatments were mixture treatment: a) 100 ppb of TiO2 NPs plus 10 ppb of a mix of organic PCPs and b) 100 ppb of TiO2; bulk plus 10 ppb of mix of organic PCPs. The organic PCPs selected were triclosan (antimicrobial), OTNE (fragrance) and benzophenone-3 and octocylene (UV filters). Bioaccumulation of inorganic compound such as TiO2 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 TiO2 and was driven by the size of the particles. TiO2 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 hydroxy groups, 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 TiO2 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 through the cell body and of the TiO2 NPs, the adsorption and some of the transformations and any of the two forms of TiO2 provoked significant (p<0.01) changes in the activity of antioxidant enzymes compared (EROD, SOD, CAT, T-GPx, S-GPx, GST and GR). Despite of higher bioaccumulation of triclosan and benzophenone-3 in the TiO2 NPs mixture than TiO2, 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 Andalucia (PE2011-RNM-7812 project). J. 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 increase 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 zinc ions (ZnCl2) or zinc oxide nanoparticles (ZnO-NP) in 2.2 cm2 soil, followed by 21 days elimination in clean soil. Two concentrations
were tested for both ZnCl₂ (250 and 500 µg Zn g⁻¹) and ZnO-NP (500 and 1000 µg Zn g⁻¹), corresponding with EC₅₀ and EC₃ₐ for effects on reproduction. Based on the measured internal Zn concentrations in the earthworms over time of exposure, the kinetic parameters, k_e – assimilation rate constant (g wet body weight g⁻¹ day⁻¹), and k_e – elimination rate constant (day⁻¹), were estimated using a one-compartment model for the effects of exposure to waterborne Zn concentrations in the ZnCl₂ treatments k_e 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 (ZnCl₂ vs. ZnO-NP) for either EC₅₀ or EC₃ₐ when related to total Zn concentrations in soil, but at the EC₅₀, the k_e related to porewater Zn concentrations was significantly higher for ZnCl₂ 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 control soil 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
M.D. Pavlik, University of Aveiro / Department of Biology; R. Morgado, University of Aveiro / Department of Biology and CESAM; S. Loureiro, Universidade de Aveiro / Biology
Meeting the world’s food demands without jeopardizing the equilibrium of ecosystems is one of the grand challenges of modern agriculture. Nanoparticles have been proposed as a solution to this problem due to their increased surface-to-volume ratio, making them attractive for widespread application. Nonetheless, increasing evidences suggest that the processes governing the bioavailability, fate and effects of nanoparticle 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 nanoparticle’s 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 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 diethroate and waterborne uptake of Ag from Ag-enriched sewage sludge and Ag ENPs in a sediment-dwelling oligochaete
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 characterize Ag ENP and Ag ENPs in natural sediment to get a better understanding of Ag bioavailability, due to populations of diethroate 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 AgNO₃. Worms were exposed in OECD-media (ISO 6341-1892) for 4h to estimate the unidirectional uptake rate constant for AgNP exposure to sediment amended with Ag ENPs or AgNO₃ 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 AgNO₃ in solution with an uptake rate constant of 8.0 ± 0.5 g⁻¹ 25h⁻¹ and 1.4 ± 0.2 g⁻¹ 25h⁻¹ for AgNP (kₑ = 0.34 Lg⁻¹ h⁻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 AgNO₃ 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 wet g⁻¹ d⁻¹ for AgNO₃ and 0.005 g wet g⁻¹ d⁻¹ for Ag ENPs. Silver from both forms was poorly bioavailable (AE ranged from 3-12% for AgNO₃ and 0.1-0.5% for Ag ENPs). Ingestion rates (IR) were significantly higher for worms exposed to Ag ENPs compared to AgNO₃ (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
S.G. Gutiérrez, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria / Environment; M. Fernández, M. Babin, INIA / Environment; A. Obrador, Universidad Politecnica de Madrid; D. González, Universidad Politecnica de Madrid / Quimica y Tecnologia de Alimentos; C. del Río, C. Garcia-Gomez, INIA / Environment
Iron oxide 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 indicators 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 calcareous soil. Effects related to the bioavailability of ZnO-NPs in different conditions were observed in all treatments. These results could be related to the increase of Zn bioavailability in acidic pH. Differences between ZnO-NPs and bulk ZnO were not observed with some exceptions. This study was funded by the Spanish project: RTA2013-00901-C02-01/02

TU118 Effects of freshly applied and aged silver nanoparticles on selected soil organisms
C. Heuck, S. Dambietz, A. Duffner, M. Erden, T. Moser, Eurofins Agroscience Services Ecotoc GmbH
Silver nanoparticles (Ag-NP) 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 AgNPs and silver nitrate (AgNO₃) as a reference on the soil invertebrate species Eisenia fetida, Folsomia candida and Hypoaspis aculeifer, LC₂⁰, EC₅₀, EC₉₀,median, and NOEC/LC⁵₀ec for both mortality and reproduction were determined. Tests were performed according to the respective OECD guidelines (OECD 222, 226 and 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 AgNO₃ 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 AgNO₃) 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 AgNO₃, 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
K. Schlich, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology; K. Händ-Rinke, Fraunhofer IME
Iron oxide nanomaterials (Fe₃O₄, Fe₂O₃) are widely used e.g. for remediation, water treatment as well as in inks, contrast agents, dietary supplements and toners.
Concentrations from Ag added either as Ag ENPs or AgNO3. Worms were exposed to environmentally relevant concentrations of Kocide®3000 (nano-enhanced copper nanoparticles) and CuSO4. Kinetic models were used to calculate Bioaccumulation Factor (BAF). Analysed for Cu-content with inductively coupled plasma mass spectrometry (ICP-MS). Kinetic models were used to calculate Bioaccumulation Factor (BAF).

A biodynamic understanding of dietborne and waterborne uptake of Ag from 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 assimilation efficiency from food, which need to be tackled for developing consistent science-based regulation. The present study was divided in two steps: (a) acute toxicity tests, exposing nematodes to six concentrations of Ag-NPs; (b) 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 Ag or AgNPs could induce alterations in sensitivity to Ag exposure, and a range of other metals (Cu, Cd and Cr, or Ce nanoparticles), and the herbicide paraquat. C. elegans were exposed to three sub-lethal concentrations of either Ag or AgNPs. Changes in the response to Ag, either ion or nanoparticulate, were measured by standard toxicity tests, exposing nematodes to six concentrations of Ag, assessing changes in development, fertility and survival. The characterization was conducted using Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM) and ICP-MS/MS. 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. Field studies are planned in two steps (applied via sewage sludge) to the soil environment. Our goal was 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 this period. In our experiment, by the Federal Ministry of Education and Research (BMBF), we have used 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 AgNO3. Here we are focussing on effects on collembolans and oribatid mites, two important components of the soil invertebrate fauna. The multigenerational 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)**

**TU1/120**

**Multigenerational Exposure of the nematode, C. elegans, to Silver Nanoparticles- Will it Change the Toxic Response?**

L. Rossbach, Norwegian University of Life Sciences UMB / IMV; E. Maremonti, M. Kleiven, P. Lebed, Norwegian University of Life Sciences UMB; D. Oughton, Norwegian University of Life Sciences / Centre for Environmental Radioactivity (CERAD CoE); D.A. Brede, Norwegian University of Life Sciences UMB. Due to their wide range of applications, silver nanoparticles (AgNPs) have become one of the most widely used nanomaterials 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 generations. 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 Ag or AgNPs could induce alterations in sensitivity to Ag exposure, and a range of other metals (Cu, Cd and Cr, or Ce nanoparticles), and the herbicide paraquat. C. elegans were exposed to three sub-lethal concentrations of either Ag or AgNPs. Changes in the response to Ag, either ion 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/MS. 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. Field studies are planned in two steps (applied via sewage sludge) to the soil environment. Our goal was 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 this period. In our experiment, by the Federal Ministry of Education and Research (BMBF), we have used 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 AgNO3. Here we are focussing on effects on collembolans and oribatid mites, two important components of the soil invertebrate fauna. The multigenerational study addresses the long-term effects of environmentally relevant concentrations. In this presentation first results of the TME study are shown.

**TU1/121**

**Investigating effects of silver nanoparticles on the soil community - An outdoor TME study**

M. Haas, S. Witz, Research Institute gaiac; J. Oellers, N. Willius, A. Fürste, S. Pfefferle, S. Miller, A. Toschki, gaiac Research Institute for Ecosystem Analysis and Assessment

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 and the disposal of waste containing Ag-NPs. The composting 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 this period. In our experiment, by the Federal Ministry of Education and Research (BMBF), we have used 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 AgNO3. Here we are focussing on effects on collembolans and oribatid mites, two important components of the soil invertebrate fauna. The multigenerational study addresses the long-term effects of environmentally relevant concentrations. In this presentation first results of the TME study are shown.

**TU1/122**

**Investigation of soil health assessment system and its application on field sites**

S. Kim, Konkuk University / Department of Environmental Health Science; J. Cho, Konkuk University / Department of Environmental Health Science; J. Kwak, S. Nam, Konkuk University; R. Cui, Konkuk University / Department of Environmental Sciences; K. Ahn, S. Jeong, Kunsan National University / Department of Environmental Engineering; Y. An, Konkuk University / Department of Environmental Health Science

The soil health assessment system is an important tool 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 evaluate sensitivity changes in soil functions, and these values were applied to indirect approach for interpolated 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) calculation 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 statistic 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 quantification of soil quality. To verify whether the KoSMAS is well operated on field sites, farm soils including metal contaminated- (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. With the decreasing sampling sites, the results of first and second samples 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 quantification of soil quality is very useful technique for soil management and reuse determination, and the method development should be carefully determined and modified in future studies. This work was supported by the Korea Ministry of Environment as the GAA Project (201400056001). This study was also funded by the Korea Ministry of Environment (MOE) as "The Chemical Accident Prevention Technology Development Project"
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 fulfill the purpose of the experiment (unmotivated behavior, well feeding, no mortality of individual bees, full comb with all brood stages). 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
S. Aldershof, Biosearch & Evaluation; F.M. Bakker, Eurofins-Mitox
In earlier work we analysed NTA field studies performed with the same active substances 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 their responses to insecticides (e.g. difference in MDD’s, PRC on variances).


TU125
Shell alterations in Lottia subrugosa as putative biomarker for multi-impacted coastal areas
F. Begliomini, Unifesp Universidade Federal de São Paulo; S.M. Almeida, Universidade Católica de Fornambuco; C.D. Seabra, Federal University of Sao Paulo / Marine Science, I Castro, Universidade Federal de Sao Paulo / Instituto do Mar 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 genetic analyses. In addition, shell 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, width and thickness of non-limacine limpets were observed along the geographical 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 the southern 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 terbutylazine 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 exposure studies. Five different scenarios, representing combinations of direct and indirect effects at higher concentration levels. 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 15N and 13C stable isotope analyses. Moreover, the fitness of the omnivorous specie 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
K. Loersch, German E; B. Bayer, German Environment Agency; R. Schaefer, University Koblenz Landau / Institute for Environmental Sciences; C. Kohl, D. Schnee, German Environment Agency; R. Gergs, Umweltbundesamt
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 pesticide mixtures 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 cumulatively 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 terbutylazine. 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 exposure) 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 sp., in the microcosms with the two highest pesticide concentrations, where these 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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SETAC Europe 27th Annual Meeting Abstract Book
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 Bioavailability Experiments (SBE) with the sorptive mixture of benzo[a]pyrene (B[a]P), chrysene (Chry), pyrene (Pyr) and naphthalene (Na) were performed with the industrially contaminated soil. As bioavailability-related phenomena, we used, for our studies, different experimental approaches 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. 49:4450–4455, 2015). However, in the biodegradation of PAHs by humic acids (Environ. Pollut. 184:345-442, 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).

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), tactic- 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. 49:4450–4455, 2015). However, in the biodegradation of PAHs by humic acids (Environ. Pollut. 184:345-442, 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, residues and implications for treatment strategies
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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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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.
large amounts of black particles (BP) as either solid aggregates of resinsified 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 96% of PAH in the sand fraction. The PAH contamination in the different granulometric fractions was directly proportional to the respective total 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 the 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 (PAHs) contamination in soil continues to be one of the biggest environmental challenges because of the persistence and carcinogenicity 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 treatment 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 Lumbrix terrestris & Eisenia hortensis. We conducted a 3 month experiment by exposing these two species of earthworms to phenanthrene (PAH) and fluoranthene (flu) in spiked soil samples. 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 chlordene (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 in 2006 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 offered in order to reduce the 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 estimated up to 5 months. Moreover, the risk assessment following the possible occurrence 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 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.

TU135 Comparison of the efficiency of in situ soil amendment and alimentary supplementation by activated carbons to limit Chlordene transfer to piglets

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Chlordene (Keponge) (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. This paper 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 passage and one of CLD need to be removed. 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 the potential to strongly reduce CLD bioavailability whereas sequestration of CLD during digestive processes via supplemented feed was not efficient.

TU136 Toxicokinetic of chlordene in small ruminants: linearity and half-life

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The former use of chlordene (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 agricultural products of livestock origin, 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 halflife 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 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

M. Guedes Laranja, Masaryk University / Faculty of Science, RECETOX; J. Hofman, Masaryk University, RECETOX / Faculty of Science RECETOX, L. Bielska,
Microorganisms play an important role in the bioavailability and fate of organic pollutants in soil when influencing their desorption from soil as well as sorption/desorption equilibrium by continuous degradation and/or production of solubilized metabolites. 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 in 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 assess DDE-bioavailability before and after sterilization. The results of the sterile and non-sterile treatments were compared between sterilization 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 CO2 in ready biodegradation tests, e.g. the headspace test (OECD 310). However, due to CO2 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 (sodium nitrate and illite clay) could mitigate the toxicity of the common anionic detergent cetylpyridinium chloride (CPC). Furthermore, we compared these OECD 310 results with similar sorbent addition studies of the OECD 301/E 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.**

Y. Jeong, KIST Europe; A. Schaeffer, K. Smith, RWTH Aachen University / Institute for Environmental Research.

Anthropogenic contaminants have been found in surface waters at concentration levels that cause concern 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 water quality at a given time always exist. Passive sampling techniques 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 defined in the WFD since these are baselined 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 (POCS) 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 KOW) of each compound. This study is intended to enhance the development 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-saharan urban rivers.**

A. Lalitte, University of Geneva / Institute for Food, V. Stalveyk, J. Pott, University of Genova / Department of environmental and aquatic sciences.

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. Cumulatively 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 spread. The aim of this study was to investigate the contamination of ARGs and the resistance profile of extending-spectra -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 (blaTEM, blaOXA-51, blaox-23, blaox-24, blaox-35 and aadD) 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 -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 pulotypes), a global resistance to Zn, Cu, Ni and Pb and a high resistance profile against -lactams (100% resistant to CFC, CEM, ATM, FEP, PEP, CMX, 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.**

M.E. Monaguth, North West University (Potchefstroom Campus) / Microbiology; C. Trouwborst, North-West University / Unit for Environmental Sciences and Management.

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 MDRI genes could be responsible for the observed efflux pump 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), Schoomspruit River (13.5%; 5 isolates) and Crocodile River (8.1%; 3 isolates). All the isolates were completely resistant to Fluconazole. Efflux pump genes were detected in most of the isolated species, CDR1 (85%), FLU1 (85%) and MDRI (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...
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 were used in this project to expose microbial biofilm communities taken from river sediment to systematic scenarios of antibiotic stress. Sub-inhibitory levels of erythromycin, roxithromycin, ciprofloxacin, tetracycline and penicillin were exposed over extended periods of time in order 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 macrocides in 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 present within agricultural and domestic run-off, macrocides 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, macrocide antibiotics were added to the European Commission’s Water Framework Directive, as they have frequently been found at high concentrations in the environment and technology. T. Schirmer, Karlsruhe Institute of Technology 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 ARG prevalence of each community and how they change over time and with different concentrations of antibiotics. Our data suggests that current environmental concentrations of macrocides do not have a selective effect on bacteria in experimental assays.

TU145 In vivo exposure of marine mussels to Sulfamethoxazole: uptake, bioconcentration and metabolization


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 the fact that these animals which enter the gill epithelium. 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 metabolism in mussels become a relevant issue due its high consumption. With this in mind, an experiment was undertaken in a marine mesocosm where Mytilus galloprovincialis were exposed to an environmental relevant concentration of sulfamethoxazole during 96h. After this period, the mussels were exposed for 24 hours under real commercial conditions, in the facilities of a shellfish enterprise dedicated to import and export bivalves for human consumption. A control group was included to demonstrate the stability of the environmental conditions. Seawater, mussel and haemolymph were sampled at different intervals 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 metabolism 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.


TU146 Persistence of the antibiotic sulframethoxazole in river water microcosms


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 in 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 sulframethoxazole 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 from the microcosms was held in experiments. Moreover, the effects of the antibiotic on the natural microbial community were assessed in terms of cell vitality, abundance and phylotogenic 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 detoxification of leaf litter, a crucial function within detritus-based stream ecosystems. However, antimicrobial substances' target microorganisms
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 absorbed 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, gammarid's 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, gammarid's 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 decompositional pressure, which may have triggered an enhanced food quality. In contrast, no diet-related effects were 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 second 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. In the Ukraine research of pharmaceuticals in surface waters has not been carried 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. And We 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) at!Using 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 taxonomic magnification factors (TMFs) under the Water Framework Directive: some practical advice on selecting and determining a TMF  
O. PERCEVAL, French National Agency for Water and Aquatic Environments / Research and Development Directorate; M.P. Babut, Irstea / Water; M.A. Bonnell, Environment and Climate Change Canada / Ecological Assessment Division; K. Borga, Department of Biosciences, University of Oslo / Department of Biosciences; L.P. Burkhard, U.S. EPA / ORD NHEERL MidContinental Ecology Division; S. Danielsson, Swedish Museum of Natural History; M.R. Embry, ILSI Health & Environmental Sciences Institute; T. Fettweis, University of Natural Resources and Life Sciences; K. Koschorreck, Umlweltbundesamt; D.C. Muir, Environment Canada / Aquatic Contaminants Research Division; D.E. Powell, Dow Coming Corporation / Environmental Sciences; C. Rauter, Umlweltbundesamt / International Chemicals Management; H. Ruedel, Fraunhofer IME - Institute for Molecular Biology and Applied Ecology / Environmental Monitoring.  
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 (EQSbiota) 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 can be considered representative of the concentrations in lower trophic levels. In contrast, the bioaccumulation of the contaminants 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 EQSbiota (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 the trophic level for a given substance is a crucial 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. Firstly, 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 amount in water. Furthermore, for each 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 WWTP effluents. 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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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 (EQSbiota) 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 can be considered representative of the concentrations in lower trophic levels. In contrast, the bioaccumulation of the contaminants 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 EQSbiota (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 the trophic level for a given substance is a crucial 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.
compound. This $K_{ow,air}$ 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.

T1/152 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. Therefore, 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 in fish 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.

T1/153 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, Lactuca sativa, Telfaria occidentalis, Allium tetracoccum and Triticum aestivum were collected from farms situated and sampled from Iyana-Iba at latitude 6.482°N and longitude 3.200°E; Baruwa Pipeline at latitude 6.594°N and longitude 3.272°E; Iyana-Osun at latitude 6.511°N and longitude 3.292°E; and Ibadan-Araba at latitude 6.514°N and longitude 3.356°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 Spectrophotometer (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 olitorius, 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. Zinc and Cu in all the vegetables of the four farmlands were lower than recommended EU standards. Zinc, Nickel, Copper, Lead and Chromium in the vegetables were lower than the Environmental Quality Standards developed from studies conducted in Iyana-Iba, while Iron and Chromium were higher. There were no statistically 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 these heavy metals in some of the farmlands are hereby presented.

T1/154 In vitro to in vivo extrapolation of hepatic metabolism in fish: update on development of OECD Test Guidelines and Guidance Document


Chemical biotransformation represents the largest source of uncertainty in chemical bioaccumulation assessments, and model-based estimates of chemical bioaccumulation in fish may be greatly improved by including biotransformation rates, as measured in vitro. In vitro substrate depletion assays, using rainbow trout hepatocytes and rainbow trout liver sub-cellular fractions (9,9), have been 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 ($CL_{int,iv}$). 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 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.

T1/155 PBDEs and legacy POPs in the Antarctic environment and biota

M. Khairy, University of Rhode Island; E. Brault, University of California Santa Cruz; R.M. Dickhut, College of William Mary VIMS; R. Loehmann, University of Rhode Island / Graduate School of Oceanography

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; (iii) determine the origin of contaminants in Antarctic soils and biota 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/lipid in Lobodon carcinophagus, Leptonychotes weddellii and Ommatophoca rossii respectively. In Orcinus orca, OCP concentrations (mean 2,112 ng/g lipid) were ~ 1 – 2.0 – 0.20 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 same species with respect to PBDE concentrations in blubber. Third, there were no clear trends evident in POPs concentrations across the seal species. Concentrations of HCBs 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, it could reflect the ability to bioaccumulate at a lower rate than other PBDEs taken up. In summary, concentrations of legacy pollutants, dominated by DDTs and chlorodanes, 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.

T1/156 Prediction of in vivo biotransformation in fish and human for the refinement of bioaccumulation

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Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; T.N. Brown, TNB Research / Department of Chemistry; L. van der Wal, OECD / Environment Health and Safety Division Environment Directorate; P. Gramatica, University of Insubria / DiSTA Biomonitoring 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 \((H_{b})\) 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 (Q SAR) represents 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 vitro biotransformation-related parameters, such as \( H_{b} \) in fish and humans. These new 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. biopersistence 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. Aim 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 ratio and the type of hemoglobin, type of hemoglobin. Statistical analyses were performed with JMP® Pro 12 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) value 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. The Haldane (M) constant presents a species obstacle. 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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TU160 Effects of DDE occurrence on the Solanum lycopersicum rhizosphere under different organic carbon sources


soil contamination: soil is a complex multi-biological system and plays a leading role in promoting the microbiological activities of selected plants to decrease pollutants from soil through different processes occurring in the

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rhizosphere involving symbiotic and non-symbiotic interactions with natural microorganisms. *Solanum lycopersicum* is known to be able to accumulate DDTs in roots, owing to exude production, which makes this contaminant bioavailable in soil. In this context, a greenhouse experiment was performed using pots filled with DDT-contaminated soil and watered with different kinds of dissolved organic carbon (DOC). Control plants (without pots) and DDT 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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Phytoremediation 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 phytomanagement 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 (biomass and sewage sludge). Results of the column bioassays showed that biochar addition is able to improve the mineralization of the 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 ~5.5. The presence of vegetation did not have any effect on the soil pH. Both experiments with 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 estimation 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 assessed in the columns, but the presence of biochar led to an increase 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 weight, although 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, in general, responsible for the eutrophication of aquatic ecosystems. Facing this reality it is necessary to develop technologies that might recover the quality and minimize the toxicity of the wastewaters before they can be released into the aquatic environment. The releases of partially treated or untreated wastewaters into water bodies are, in general, responsible for the eutrophication of aquatic ecosystems. Facing this reality it is necessary to develop technologies that might recover the quality and minimize the toxicity of the wastewaters before they can be released into the aquatic environment. Therefore, we concluded that 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 being financially unfeasible are extremely impacting to the natural systems.

TU163
Evaluation of the sulfonamide antibiotic persistence in the absence of the aquatic plant Lemna minor

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, sulfaethoxazole (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*, 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 the 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 technique. The effects of the antibiotic on the natural microbial community were assessed in terms of cell viability, 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
R. Pasosad, IBNAS CSIC / Agroquimica y Conservacion del Suelo; J. Ortega-Calvo, Instituto de Recursos Naturales y Agrobiologia / Agroquimica y Conservacion del Suelo

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 fraction. We used different bacterial strains (natural microorganisms and microbially-produced biosurfactant), 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 14C-labelled PAH to the samples allowed the measurement of mineralization (production of 14CO2) 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 14C and had been maintained under the same conditions. In addition, we used desorption methods, well represented by Tranex 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 standard tool to assess the potential of slowly desorbing PAHs (Environ. Sci. Technol. 48: 10869–10877, 2014; Environ. Sci. Technol. 45: 3019-3026, 2011). In this way, we could identify situations including different degrees of bioavailability: from heavily polluted soils containing highly bioavailable compounds, to soils previously treated with bioremediation, showing a reduced bioavailability. Our results do not show a clear difference in the potentially mineralized or totally 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 C. Lutterbeck, University of Santa Cruz do Sul / Graduate Program in Environmental Technology; F.V. Zettl, P.L. Machado, University of Santa Cruz do Sul / Graduate Program in Environmental Technology The establishment of appropriate water and wastewater management systems is a vital issue, especially in developing countries. Sanitation data from these countries reveals serious hygiene, health, economic and social implications that highlight the urgent need to develop less expensive, more efficient and easy-to-install technologies which may suit real regional realities. In this recent study, we 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 photo-reactors. Hydranema grumosa was selected as the macrophyte. General characterization parameters of the wastewaters included COD, BOD, NTK, N-NH4 and total P analysis. Ecotoxicity assays were performed using Daphnia magna, because the cytoxicity, 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 BODs. Additionally, 97% of the TKN, 100% of the N-NH4, 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 cytoxic 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 showed that it 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 enable the water use even for less noble uses, such as sanitary discharges.

TU166 Biochars and compost for in situ remediation of diffusely contaminated sites G. Sigmund, University of Vienna / Department of Environmental Geosciences; C. Poyntner, University of Natural Resources and Life Sciences / VIBT EQ Extremophile Center, Department of Biotechnology; M. Kah, University of Vienna / Department of Environmental Geosciences; G. Pitaru, University of Natural Resources and Life Sciences / VIBT EQ Extremophile Center, Department of Biotechnology; T. Hübner, T. Hofmann, University of Vienna / Department of Environmental Geosciences Highly polluted areas (hot spots) are commonly remediated by excavation or other in situ remediation methods. One sustainable and conservative approach is to use biochars and composts for diffusely contaminated soils. The excavation, removal and waste disposal of tackle such contaminated areas would be disproportionately expensive. Biochar, a product of biomass pyrolysis, can be used to treat such low level contaminations by immobilizing contaminants and participating in the ecological stabilization of such sites. An innovative approach involves the use of biochars in combination with surfactants to immobilize contaminants or promote 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 were set up investigating parameters 1) for desorption of a wide range of organic contaminants (neutral and acidic) to biochars and 2) 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 these parameters are combined with lipophilicity descriptors for the organic contaminants (R empt and D low for the empty and low axial lipophilic character, 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) biochars can be used to immobilize PAHs and VOCs in contaminated soils 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. Hübner, and T. Hofmann, Bioresearch. Technol., vol. 214, pp. 225–233, 2016. [2] G. Sigmund, H. Sun, T. Hofmann, and M. Kah, Env. Sci. Technol., vol. 50, pp. 3641–3646, 2016.

TU167 Degradation of estrogen hormones by fungi in wastewater P.G. Silva, Universidade Federal do ABC / Centro de Ciências naturais e Humanas; E.M. Leal, L.C. Rodrigues, UFABC / CCNH; V.V. Vitali, Instituto de Botânica / Micologia Aplicada 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 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 estrogens, 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 estradiol. 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 analyses 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 Estradiol standard was observed in the enzymatic production of the fungus. When comparing the degradation 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.
CHALLENGES IN ASSESSMENT AND MANAGEMENT OF COSMETICS AND PERSONAL CARE PRODUCTS (P)

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 tar 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 screening study was conducted to determine the VSRL sludge which was selected 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., KH₂PO₄, K₂HPO₄, Na₂HPO₄, 2H₂O, NH₄Cl, MgSO₄, 7H₂O, CaCl₂, 2H₂O, FeCl₃, CoCl₂, 6H₂O, H₂BO₃, Na₂MoO₄, 2H₂O, CuCl₂, 2H₂O, ZnCl₂, NiCl₂, 6H₂O, FeCl₃, 4H₂O, MnCl₂, 4H₂O and Na₂SO₄ 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, KH₂PO₄, Na₂MoO₄, 2H₂O, MnCl₂, 4H₂O, Na₂SO₄, NiCl₂, 6H₂O and CaCl₂, 2H₂O 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 carbazole carboxyl with inoculum was observed based on controls, hence initial carbazole concentration was corrected before determining %carbazole degradation. Three media components, i.e., MnCl₂, 4H₂O, NiCl₂, 6H₂O and CaCl₂, 2H₂O had the most significant influence on carbazole degradation. Carbazole degradation increased from 37.63 % ± 5.18 % in unoptimized media to 58.44 % ± 9.39 % in the optimized media which contained MnCl₂, 4H₂O at the high level and both NiCl₂, 6H₂O and CaCl₂, 2H₂O 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.

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, tsh/gf gene was significantly down-regulated by benzophenone-2 and -8, suggesting their thyroid disrupting potential through hypothalamic-pituitary-thyroid axis. Our in vitro observations show that benzophenone-1, -2, -7 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

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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. methylbirdroto glutaronitril or formaldehyde), phthalates (e.g. dibutylphthalate), peroxides (e.g. sodium perborate), aromatic hydrocarbons (e.g. anilinophenol), phytotoxins (e.g. Aconitum napellus, or Strophanthus), glucocorticoids, phenylenediamine, acrylates, N-nitrosodisedynamoline, ammonia, thiglycolic acid, methanol, or diethylhexyl 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 Environmental 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 assess 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 of 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 C. 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 ng/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 physico-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-filer. 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 determined in parental generation. Scootal and copulating fertility 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 framework requires, among other conditions, environmental risk assessment (ERA) analysis to determine the level of risks for the environment. The data protection for wildlife and other biological protection products. The relevance of 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 dependent on the sorbant, the most important one is the amount of organic matter in the soil. 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 Kwm (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 in soil depth, soil clay content and clay type may change with time and may influence the results. 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 exposure. The refinement option comprises a conservative application strategy for assessing surface water exposure (6 drainage and 3 run-off scenarios) and groundwater exposure (1 scenario) and is intended to provide a realistic worst case assumption 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 vary. This study considers a zonal approach particularly useful for planning the future use of these scenarios in the context 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 animal husbandry


The transformation of veterinary pharmaceuticals and biocides onto animal husbandry 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’) 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 regulatory 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, developed and refined 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 order to facilitate collaborative international work and to secure the 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 aerobic 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 parasitic insect, Myrmeleon formicarius. 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 endoparasitic faunulae 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 different effects across sites on: (1) insects breeding in dung of treated animals, (2) trophic 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 different approaches to measure them in the field. Experimental 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. F. Blanckenhorn, E. 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. A formal study to develop a higher-tier, modelling-based, approach for the assessment of the environmental impacts of bath treatments, 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_{water}), which are subsequently compared with Predicted No Effect Concentrations (PNECs) derived from environmental effects studies. PEC_{water} 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 underestimation of exposure and to the divergence between predicted and observed exposure patterns in the environment. In this paper, we discuss higher-tier refinements for the calculation of PEC_{water} 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 ecosystem is the particulate matter that chronically leave metals available in surface waters for uptake by aquatic organisms and plants. Moreover, lead isotopes ratios found in sediment had a 50+25 μg/L of Cd and 250 μg/L of Cu. The latter is the upper limit of the threshold concentration of 0.1 µg/L. The latter is the upper limit of the (PEC_{water} predicted threshold 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_{water} 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 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 in the water column) has 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 also for the food additive, antibiotic production, biocides and other chemical substances under REACH legislative framework.

TU181
SETAC Interest Group on Dung Organism Toxicity Testing
J. Roembke, ECT Ökotoxikologie GmbH

The Aquatic and Terrestrial Environment (P)
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 in the marine environment due to the potential toxic effect 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 biomarkers. 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 Ptb>Cu>Fes>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 among the stars 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 (hydrolysates constant) in sea-water.

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 bioic 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 CaCO3), 6.0 to 9.3, and 0.05 to 4.9 mg/L, respectively. The chronic toxicity database we used included NOEC and LOEC 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 percentile of SSD (HCS) 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 mg/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.

The investigation of the ecological vulnerability of aquatic organisms for cadmium

J. Park, S. Kim, Gwangju Institute of Science and Technology; W. Kim, J. Yeom, T. Jeong, Gwangju Institute of Science and Technology / School of Earth Sciences and Environmental Engineering

Although the traditional risk assessment approaches have played a key role, the extrapolation from the initial response to ecosystem response and the exclusion of interaction among the organisms are still critically assessed. Investigating 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 threat to the species. As a result of an 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".

Molecular Toxicity of Metal - Mixture and Defense Mechanisms in Zebrafish (Danio rerio).

W. Meertel, University of Antwerp, Dept. Biology; K. Smeets, Hasselt University / Centre for Environmental Sciences; R. Blust, University of Antwerp Faculty of Sciences

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.

Binary metals mixtures effects on spring barley Hordeum vulgare nutrient 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 considerably influenced by the interaction among the metals. Analysis of variance showed that exposures in a single and combined metal 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.

Cu and Cd affect distinctly the biochemistry of two ubiquitous tropical green algae

P. Echeveste, Pontificial Catholic University of Chile / Department of Ecology; J. Carmo Silva, Universidade Federal de São Carlos / Department of Botany; A. Claudino dos Santos, A. Lombardi, Universidade Federal de São Carlos / Botany Department

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 phytoplankton 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 proportion 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.

TU192
Limited solubility of test items in regulatory honey bee (Apis mellifera) testing: potential use of solvents, solubilizers and viscosifiers with aqueous sugar solution

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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 possible dietary exposure pathway for 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 LC50/LD50 (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 LC50/LD50 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 in order to 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®) and one solubilizer (sodium lignosulphonate (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 trigger values. 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 the end of October and ended in mid April in 2014. From May onwards, several flowers were detected: a) upon tagging, b) when they left the hive and c) when they entered the
hives. 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. Görlisch, WSC Scientific GmbH

Lower tier risk assessments of plant protection products for bumblebees 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 for the following endpoints: 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 technical 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.

**TU195**

**Experiences with made with bumble bee testing and the antrhnic diamide insecticide, cyantraniliprole**

A. Dinter, DuPont de Nemours (Deutschland) GmbH / Crop Protection; A. Samel, DuPont de Nemours (Deutschland) GmbH

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 antrhnic 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 adult 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 latest 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 LD50 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 dimefox 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

M. Allan, Atlantic Pollination Ltd; R. Dean, Red Beeive Company

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 will discuss the following endpoints: 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 number 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 technical 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. Fricker, Eurofins Agrosciences Services Ecotox GmbH / Ecotoxicology Field; O. Klein, Eurofins Agrosciences Services EcoChem GmbH / Ecotox Field; S. Knaebe, EAS Ecotox GmbH / Ecotox Field

Bumble bees (*Bombus terrestris*, *Lympeoponera, Apidae*) provide important pollination services and are therefore used commercially (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? 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 indicator. Concerning the switch-points, 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**

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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.
TU200
On the way to a new guideline - Results of three years of bumble bee semi-field testing
A. Alschler, 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 working group of 2015 and 2016. Experiences were gained regarding different crops, season and size of crops. Mortality, flight activity and survival 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.

TU201
Experimental design for semi-field trials with solitary bees: Status quo after three years of development
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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 working group in 2015 and 2016 a semi-field test design for solitary bees was developed. First semi-field tests with the red mason bee (Osmia bicolor L.; Hymenoptera, Megachilidae) were conducted in 2014 with different set-up and release techniques to develop a method with high matching 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. bicolor 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. bicolor. Additionally, the statistical analysis proved 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 bicolor (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. bicolor was developed, which is based on the EFSA document, testing principles for semi-field studies (EPPO TR 20010) and the outcome of an ICPR 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. bicolor (cornuata) was the reduction of 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 (Kniabe 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 fenoxycarb treatment. The concentrations of the test item fenoxycarb (150 and 350 g a.s./ha) was sprayed into the bee nests. A single stressor treatment group, with 4 replicates, i.e. tunnels with 90 m² of flowering Phacelia. Nesting units with cocoons of both sexes of O. bicolor 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. bicolor in Phacelia. Finally, the question is discussed whether fenoxycarb is a suitable reference item for semi-field studies on O. bicolor.

TU203
Analysis of the effects of thiamethoxam in the brain of africanized honey bees through MALDI-imaging technique
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 thiamethoxam, an neurotoxic insecticide widely used in Brazil. Based on the importance of toxicological studies on the evaluation of possible harmful effects that the substances can have on the brain of bees, the aim of this study is to evaluate 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 LC50/100. 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-TOP-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 compared 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 a 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 0.3. In effect a substance is considered low risk if the LDD50 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) which in mathematical terms a value of 0.03 only meets the SPG for a given LDD50 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 LDD50 is not technically possible due to low toxicity and/or limited solubility (e.g. many pyrethroids and fungicides). In these cases (where no observable effect dose (OEED) and 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 1/3 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. LDD50 or NOEDD) and the ceiling/tier of protection afforded by a given exposure toxicity ratio (ETR) can be calculated allowing for

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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., 2010) 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: 371564090; funded by the German Federal Environment Agency) recommendations for risk mitigation measures which might be applicable 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 key yet partially specific characteristic 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 ecosystem/human health and the damage on the resource itself without double counting impacts. This study provides insight into the problems of considering freshwater in an assessment as an AoP natural resource. 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 elements for 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 presence 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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Protecting terrestrial ecosystems of which 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 estimation of mid-term water scarcity 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, C_{PWS,TI}. 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

L. Scherer, VU University Amsterdam; S. Pfister, ETHZ / Chair of Ecological Systems Design

Water Newly 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 increased the spatial resolution from watershed level to 0.5 degrees to account for local water scarcity. We analyzed a multi-stressor dataset to assess uncertainties based on latent 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 increase was, including both surface and groundwater resources, severe and 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 water scarcity. On the other hand, flow happen at a local scale. To test the approach, an integrated land use model is followed to carry out water-related performance, taking into account both water consumption does. With regards to carbon footprints, CO2 emissions contribute
implied changes in the reservoir reservoirs. The analysis of the results shows that the reservoirs alleviate water scarcity. On the other hand, flow happen at a local scale. To test the approach, an integrated land use model is followed to carry out water-related performance, taking into account both water consumption does. With regards to carbon footprints, CO2 emissions contribute
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 within 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 biophysically quantified at midpoint level and monetized at endpoint level. Next to presenting and discussing the developed method's validity, potential applications and possible further improvements relate to a regionalisation of more impact categories, and adding more impact pathways. 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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In line with this, the UNEP-SETAC land-use group recently pointed out an actual reference situation when modelling land use changes interventions. In details, the advanced methodological framework could be proposed for the final CFs, increasing their usefulness.

TU216

How Sustainable is Hydropower Really?

L. Scherer, VU University Amsterdam; S. Pfister, ETHZ / Chair of Ecological Systems Design

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 2010 and 150 globally distributed hydropower plants, covering a wide range of global annual electricity generation. Since a reservoir can fulfill multiple purposes, impacts were allocated to hydropower based on its ranking among all the purposes the reservoir fulfills. 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 generalised linear modelling. About 100 records were available as training data, from which the empirical hydrological regions were calculated. The 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 when cause larger impacts in comparison with water consumption does. With regards to carbon footprints, CO₂ 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 m³ H₂O/GJ, and the carbon footprint is 76 g CO₂/MJ. However, both vary considerably across nations and even more on individual plant level. Therefore, a more uniform approach is needed, which can be achieved by considering potential impacts of hydropower on water stress.

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 addressing impacts of water consumption on ecosystems showed that available methods cannot or only partially be compared and combined within a consistent framework (van der Brink 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 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 of indicators organized 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.
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).

TU211 Making biotic resources count in the LCIA framework
E. Crenna, European Commission - Joint Research Centre / Directorate D - Sustainable resources, Bioeconomy Unit; S. Sala, European Commission - Joint Research Centre / Bioeconomy unit

Nutrient resources provide a natural capital stock, 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 required, 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 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, we suggest both the potential and the required indicators to evaluate the depletion of biotic resources in the LCA context and support the transition to sustainable bio-economy at global scale.

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
M. Berger, Technische Universität Berlin / Chair of Sustainable Engineering - Offenbach ZT; T. Steiner, ETH Zurich (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, and inorganic 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 what strategies and development priorities will be focused 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.

TU222 Estimating environmental impacts of mixed dishes: A case study on pizza
A. Zabeo, University Cà Foscari Venice; C. Bellio, Contarina SpA; E. Giubilato, L.B. Pigatin, IQSC-USP / Instituto de Química de São Carlos  IQSC; R.R. Rachide, TU228

The development and application of a new characterisation method for enhanced exposure to Naturally Occurring Radioactive Materials (NORM) P.J. Joyce, KTH Royal Institute of Technology; A. Gornovskii, A.H. Tkaczuk, University of Tartu; A. Björklund, KTH Royal Institute of Technology Norway, 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 increase human exposure to artificially increased levels of naturally occurring radionuclides. For example, coal contains low levels of 232U and 232Th. 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 patented 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 source of human health (which contributed 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 LCIA?

P. Martz, L'Oréal Research & Innovation / LORÉAL; M. Philippe, L'Oréal Research & Innovation; J. Lharridon, L'Oréal Research & Innovation / Life Sciences Direction

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'Oréal were renewable. This represents around 1,400 ingredients from more than 1,000 species which are used in more than 100 formulas. 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-toxic raw materials. On the other hand, Life Cycle Analysis (LCA) is a powerful multicriteria tool to assess the different potential environmental impacts and services. 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 furan dicarboxylic acid from sugar beet cultivation

M. Garcia Gonzalez, Politecnico of Milan / Dipartimento di Chimica Materiali ed Ingegneria Chimica G Natta Politecnico di Milano; P. Borjasson, L. Björnsson, University of Lund / Environmental and Energy Systems Studies; R. Hattf-Kaul, University of Lund / Environmental Chemistry Laboratory

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. Polymers, containing both aliphatic and aromatic monomers, are one of the most promising families 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 based on “cradle to gate” assessment and System boundaries 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 succrose, the conversion of succrose to fructose, the conversion of fructose into 5-hydroxy methyl furfural (HMF) and finally the production of FDCA from HMF. The whole process is 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 kg CO₂/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 kg CO₂/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

A. Zabo, University Ca Foscari Venice; C. Bellio, Contrinara SpA; E. Giuliabili, L. Pizzol, University Ca Foscari di Venice; P. Cremona, Contrinara SpA; E. Semenzoni, Ca Foscari University of Venice / Department of Environmental Sciences

Innovations and Informatics

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 or service (IPCC 2007). In this case study we used to analyze and assess the environmental impacts of the resources used for the collection of municipal solid waste by the company Contrinara SpA. Contrinara 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, Contrinara 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 PRÉ and the data were generated with eco-invent 3.1. The approach, based on cause-effect chain from inventory flows to areas of protection. By running simulations according to different global to regional socio-economic and land use patterns in developed and developing countries are generating concerns about their dependence and building a more sustainable future through the use of polymers. Furthermore, we do not always have the whole dataset in databases necessary to assess in details our raw materials, which are commonly used and how their profiles are compared to chemicals from synthetic or petrochemical origin.
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 PBT/vPvB 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 fate of the 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 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 Organisational 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. One of the most comprehensive regulatory approaches for assessing the ecomamochemical risks of substances is 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 (Kow), bioconcentration factor (BCF), biomagnification factor (BMF) and the total elimination half-life (HET). 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, HET, 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.
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 305), 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 studies in both in vivo and in vitro conditions (e.g., OECD 305); and the BMF, bioaccumulation factor (BAF), and the bioconcentration factor (BCF), biomagnification factor (BMF) and the total bioaccumulation classifications (e.g., “B” or “not B”). The BAT seeks to facilitate decision-making in multi-stakeholder processes and is intended to provide a weight of evidence for regulatory decisions. The revision of the ECHA guidance on PBT assessment of chemicals (Chapters R.11, R7(b), 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-persistence under realistic conditions, should not be used. A very conservative 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 extent 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 is not elaborated upon to guide for identification of bioaccumulation 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 Tonage Chemicals and Implications on the Identification of PBT/PrvPvB-Substances


‘No Data No Market’ is the slogan of the European Union’s regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [1]. No substances that are produced or imported into 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 by only 5% of the registration dossiers. With the aim to evaluate systematically the data availability 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 tree software for data on substances in regulatory data from several OECD 317 studies and performance of correlation analysis between soil/surface-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 PCB 125. Proposal for harmonization criteria for bioaccumulating 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 Kow, log Koc 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 Corg-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-depuration residues at the end of the elimination phase are discussed.

TU235 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, R7(b), 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-persistence under realistic conditions, should not be used. A very conservative 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 extent 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 is not elaborated upon to guide for identification of bioaccumulation 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.
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
A. Wiemann, German Environment Agency / UBA / Section IV Environmental Risk Assessment of Pharmaceuticals; I. Prutz, German Environment Agency / UBA / Section IV 2.2 Pharmaceuticals; J. Schönfeld, German Environment Agency (Umweltbundesamt) / IV 2.2 Pharmaceuticals Persistent, bioaccumulative and toxic (PBT) substances or very persistent and very bioaccumulative (vPvB) properties are compounds with hazardous properties. The non-biodegradability (persistence) and high accumulation in organisms (bioaccumulation) may elicit long-term adverse effects in the environment.

Identification of relevant metabolites and their toxicological properties are not considered in benefit-risk assessment for human medicinal substances. This means, identified PBT properties have no consequences for the assessment 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 definitive classification as PBT is not possible. The candidates by far are mainly pharmaceuticals 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.

The present poster shows the results of the PBT assessment for veterinary medicinal products included in the benefit-risk assessment. 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
A. Gerloff-Elias, Knooli; A. Szymoszek, DR. KNOELL CONSULT GmbH
Under Regulation (EC) No. 1907/2006 (REACH) a conclusion can be drawn that a substance fulfills 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 BBDD, 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 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 (PnMCs) 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-modelling. Furthermore, in Europe chemicals have to be registered by the manufacturers under the REACH legislation. In this process chemical property data have to be considered to the European Chemicals Agency (ECHA), besides other information. We have therefore looked at 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 proposed trigger values were used and compared to existing and newly developed QSAR models; 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].

TU240
Screening for emerging persistent and mobile organic water contaminants by liquid chromatography and supercritical fluid chromatography coupled to mass spectrometry
S. Schulze, Helmholtz centre for environmental research - UFZ / Analytical Department; T. Meier, P. Prabužík, H. Paschke, C. Bauer, Helmholtz Centre for Environmental Research - UFZ, T. Reentsma, Helmholtz Centre for Environmental Research / Department of Analytical Chemistry, U. Berger, Helmholtz centre for environmental research - UFZ / Analytical Chemistry

The release of persistent and mobile organic chemicals (PnMCs) into the aquatic environment is a risk to the quality of water resources. As a consequence of their high polarity, PnMCs 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 detection, but we assume that their number is much larger. The aim of this work was to screen for hitherto unrecognized PnMCs 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 PnMCs 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+, SupelcleanTM ENVI-Carb) before separation on Oasis WAX, ISOLUTE® ENV+, SupelcleanTM ENVI-Carb before separation on Oasis WAX, ISOLUTE® ENV+ and SupelcleanTM ENVI-Carb. Before separation on Oasis WAX, ISOLUTE® ENV+ and SupelcleanTM ENVI-Carb. Before separation on Oasis WAX, ISOLUTE® ENV+ and SupelcleanTM ENVI-Carb. Before separation on Oasis WAX, ISOLUTE® ENV+ and SupelcleanTM ENVI-Carb.
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 irreversible. If in addition they also fulfill 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 showed 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 and public health risks 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

M. Gabarron, A. Faz, Universidad Politecnica Cartagena; J. Soriano Dílisa, Universidad Politécnica de Cartagena; J.A. Acosta, Universidad Politecnica Cartagena

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, 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) but both soil and road dust. This kind of particles has an inhalable size increasing 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 food, 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

V. Musiec, Universidad Autonoma Metropolitana / Basic Sciences; M. Vaca, Universidad Autonoma Metropolitana / Energy; J. Santos, Universidad Autonoma Metropolitana; V. Domínguez, Universidad Autonoma Metropolitana / Basic Sciences

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 and 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 64072 to 36734, 239 to 83, 128, 67 to 95, 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

K. Cheung, CODA-CERVA / Trace Elements; N. Waegeneers, CODA-CERVA / OD Chemical Safety of the Food Chain; A. Ruttens, CODA-CERVA / Trace Element Service

In contrast with the high number of studies reporting Arsenic (As) specification data in various unprocessed food items, food processing studies are limited to few matrices and specification 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 specification 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. Blaivas, 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 choices. 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.cseh.ac.uk/) has monitored long-term trends in environmental Hg concentrations in birds and predation risk 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 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 showed 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

R.A. Kolind, Aarhus University / Department of Biscience - Arctic Research Centre; R. Dietz, Aarhus University / Bioscience Department; P. Bjerggaard, University of Southern Denmark / Biology

Mercury is biomagnetically bioaccumulated through 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 being stable in each sample cut into segments. 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 ug Hg g⁻¹ - with most samples in the range 5 to 10 ug 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, methymercury and inorganic mercury (Hg⁰) 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 132 mg total mercury and in a full molt, they could deposit between 39 and 63% of their total body mercury burden. The concentration 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 Hg⁰ were calculated to between 32 and 127 mg Hg⁰⁻¹.

TU249

Trees and microbes at a Hg contaminated chlor alkali facility

M. Chalu, M. Assad, A. Durand, J. Foulon, F. Maillard, C. Zappelini, Université de Bourgogne Franche-Comté

Large dumps that are used to store industrial tailings can generate significant environmental surface Hg contamination. The contamination can accumulate to such a degree that vegetation becomes biologically infertile. Vegetation 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 incorporation of Hg into perennial tissues, that were located near the dump, and that may serve as reliable indicator of past Hg exposure. We further demonstrated that most of the Hg detected in the aboveground parts of Silicicaceae 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 re-colonized the sediment of a chlor-alkali tailings dump after sediment deposition had ceased, and the microbial communities from a range of industrial microbiomes. These studies provided 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 in biofilms, notably the influence of the biofilm composition. To that end, biofilms were colonized on artificial substrata in the Veroix 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 Hg during 72 hours using microcosms. 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 thios (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 compared to the surface biofilms. The Hg concentrations in biofilms were modelled 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.

TU251

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 found in soils 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 Cr₂O₃ and left stabilizing for 3 weeks. Healthy and challenged E. fetida earthworms of similar size (400 ± 100 mg fresh weight) were kept in density and introduced in the test soils, maintained at 19 °C, in constant humidity and light conditions. For the ATT mortality and weight loss followed a dose incremental trend from 40 mg Cr/kg onwards. Mortality and weight loss were measured (14 d) in order to obtain LC₅₀ and EC₅₀ values. Cr was quantified in soils (EPA 3051A method), lxiavates (soluble fraction, DIN 38414-4) and earthworms (n=15) by AAS (SGiker General Service; UPV/EHU).

Coelomocytes were extracted from exposed earthworms (3 pools of 5 individuals per treatment) and their number and cell viability were calculated. Cr concentrations in lxiavates 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 was calculated as 97.3 mg Cr/kg, a value corresponding 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 (R² = 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 BECAR (Program of scholarships for Argentinean professionals).

Science communication and citizen science - strategies for successful stakeholder engagements (P)

TI/253

The role of citizen science in monitoring small-scale pollution events

K. Hyder, Cefas, S.R. Wright, Cefas Lowestoft Laboratory; M. Kirby, J. Brant, Cefas

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 and monitor these events, but over these 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.

TU/254

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 really 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 also research is so closely associated with public opinions, fear 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 worrisome new media. Broken in 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.

TU/255

THE MOST DANGEROUS CHEMICALS IN PRODUCTS FOR CONSUMER PROTECTION – A CONSUMER STUDY

J. Mitic, Institute of Chemistry, Technology & Metallurgy / Department of Chemistry; K. Kriminulovic, J. Randeljovic, ALHem - Safer Chemicals Alternative; M. Crucić, Faculty of Pharmacy, University of Belgrade, ALHem - Safer Chemicals Alternative; L. Sojic, V. Mart, ALHem - Safer Chemicals Alternative Modern chemicals management system was introduced into the Republic of Serbia in 2009 with the adoption of the Law on the 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 distributors 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 znati!” 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 project of campaign provided 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 level of interest 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 latter results indicate insufficient level of awareness of 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.

TU/256

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.

TU/257

Tox on Tap: Engaging the community in science

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Tox on Tap (ToT) is a five-year Community Engagement project that aims to increase the awareness of current and international 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 Philosphique 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 Soci 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.

TU/258

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 ERA a complex problem. New methods, such as mechanistic models, have been introduced [1] in higher-tier
Advancing science and application of planetary boundaries and related ecological limits concepts to enable absolute sustainability assessments (P)

TU2/62
Ecological Public Health and DPSEE – 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 DPSEE Model (Corvalan 1996), a generic representation linking environmental “states” and their determinants to individual “exposure” and health “effects,” the modified DPSEE 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 (d)PSEE model (Reis 2015) explicitly recognised multiple routes to health and wellbeing, both through direct interactions and through ecosystem services, thus bridging health with Environmental Impact Assessment. The eDPSEE 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 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 “effects,” the modified DPSEE 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 (d)PSEE model (Reis 2015) explicitly recognised multiple routes to health and wellbeing, both through direct interactions and through ecosystem services, thus bridging health with Environmental Impact Assessment. The eDPSEE 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 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 “effects,” the modified DPSEE 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 (d)PSEE model (Reis 2015) explicitly recognised multiple routes to health and wellbeing, both through direct interactions and through ecosystem services, thus bridging health with Environmental Impact Assessment. The eDPSEE 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 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 “effects,” the modified DPSEE 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 (d)PSEE model (Reis 2015) explicitly recognised multiple routes to health and wellbeing, both through direct interactions and through ecosystem services, thus bridging health with Environmental Impact Assessment. The eDPSEE 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 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

TU259
Institutional entrepreneurship for advancing toxicogenomics in ecological risk assessment: an interdisciplinary approach to bridging the gap between scientists and regulators
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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 and/or 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

TU264
Searching for the right number: which are the most suitable reference values to be compared to the planetary boundaries?

230
SETAC Europe 27th Annual Meeting Abstract Book
The growing concern around sustainability and environmental impact of products has brought a drive 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.

The aim of this paper is 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. In this paper, environmental boundaries, planetary boundaries, life cycle assessment, normalization in life cycle assessment methodology, and the resulting footprint results, e.g. claiming environmental sustainability when only a fraction of material use cradle to grave. Overall gains in space and diverting recyclables from landfill 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 and indicators 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.
flora range biomass O Supply Energy & Resource Viability Viable Supply
biology
rate, sensitivity to toxicants, and good reproducibility compared with those of other developments of (Bjørn and Hauschild, 2015) who proposed a method of biodiversity loss are not sustainable. Indeed, some normalized pressures associated test results. Further findings resulted in a discussion of the sensitivity of different aqua-Marine microalgae, as primary producers at the basis of aquatic food webs, can be indirect test species of interest changed from Toxicity Test the test species of interest changed from toxicity to two marine microalgae? Myriophyllum spicatum was tested with the emerged form under submerged conditions. However, M. aquaticum was tested with the emerged form under submerged conditions. However, M. aquaticum is a semi-aquatic species which has 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. Gesing, Eurofins AgroScience Services Ecotox GmbH / Aquatic Ecotoxicology

A ring test with Glyceria maxima is currently being discussed. In contrast to Myriophyllum 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 already been developed. One result of these tests was that Glyceria demonstrated different growth rates between different labs and also when comparing different test results 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 Glyceria 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. Gesing, 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 adaptations 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 the herbicides’ bioavailability and toxicity on organisms. Therefore, the present study aimed to investigate the possible influence of natural DOM on the toxicity of three herbicides (ibuprofen, diclofenac and flurbiprofen) in two marine microalgae, Chlorella vulgaris and Scenedesmus quadricauda. The microalgae were exposed during 6 days, in triplicates, to each herbicide alone (0.05 and 0.5 µg L⁻¹; 60.05 and D0.5 µg L⁻¹; 60.05 and 0.5 µg L⁻¹) and to mixtures with (M1: 0.05+D0.5 and M2: 6.05+D0.5+M0.55). Experiments were run with and without DOM. The effects were assessed on growth rate (µ), photosynthetic efficiency (Fv/Fm), and related lipid content (FL1;FL2;FL3;FL4). The biological and chemical environments (bacteria, herbicide concentrations, DOM quantity and quality) were changed. When no DOM was added to the cultures, the exposure of C. vulgaris to 0.05 and M2 significantly decreased µ and FL1,FL2, FL3, each at similar levels (around -59% and -50%, respectively) and Fv/Fm (around -35%), as compared with controls. With DOM addition, lower significant effects were detected on FL3 (152.25%) when the effects on µ and Fv/Fm were almost the same (about -50% and -50%, respectively). In the presence of the herbicides, 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 Φµ while the effects on FL1_tst were decreased. These results may partly be explained by a possible combination 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 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 community, 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 macroinvertebrate abundance and communities, the main effect was on algal biomass and community structure and on leaf litter processing. Arable streams had higher algal biomass and 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 microalgae 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 microalgae *Chlamydomonas reinhardtii* and the macrophyte *Elodea nuttallii* were exposed 2h in 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 5485, and 8790 deregulated genes (DG) for the microalgae and for the macrophyte exposed in the most contaminated site, respectively. Transcriptional 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. Transcriptional 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. Apip, SéE 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 consequence of human impact on water, soil and air, is 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 bioassays analyzes is using 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 tasks of assessing the ecological burden of the environmental pollution. 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 tests. 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 insecticide, 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 that determines the growth rate for the generated 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 fulfill OECD test validity criteria over a 72h test period. An EC50 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 algae 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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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, new interest has been shown in methods that predict the transcriptome 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 for the quantification of metabolic pathways. We will use FBA to establish an AOP model that represents the relationship between inhibition of aromatase and sterility at 25°C which enables single generation study over its whole life cycle. Chronic radiation 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 mg·L⁻¹ reaching 3.3 Gy and group 2 - irradiation from eggs to "old" stage (19 days) with 3 mg·L⁻¹ reaching 0.06 Gy. Liver and a protein carbonylation were followed in both groups. Protein carbonylation level was measured with in situ biomolecular assay and the nature of carbonylated proteins was done with Oxidative Damage. Carboxylation 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 differences, the time span prolongation after 7 mg·L⁻¹ exposure for 19 days compared to control can indicate that low dose rate of irradiation created better antioxidant protection than 50 mg·L⁻¹ for 3 days compared to control. By studying the effects of oxidative damage and exposure to chronic radiation, the contribution of 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 antioxidant contribution to lifespan extension. Key words: C. elegans gdp-1 mutant, chronic gamma irradiation, aging, carbonylation.
animals, which might cause hypermetamorphosis 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 multi-generational exposure-effect quantification but also exhibit another layer of observation by combining OMICS-epigenetic-behavior in complex mixture stressors with real field water. In 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 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 a liver 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
E. Trantopoulos, V. Boti, T. Alibinis, University of Ioannina / Department of Chemistry

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 extracted from a healthy volunteer (0.1 g) digested in NaOH (80 °C, 24 h) and subsequently pH was readjusted to 9.0-10.0 with HCl. Then, the sample was mixed and dispersed using Aluminium oxide and MgSO4. 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 MgSO4 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% NH4 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 (R²). The method was shown to be in terms of reliability 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 post-mortem samples (of suicide and overdose cases). There were no histopathologic alterations and differences in the number of apoptotic bodies between the groups. K67 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).

WE010
Cytotoxic and genotoxic effects of contaminants present in a highly polluted estuary in multiple organs of rats
C.M. Cardoso, Federal University of Sao Paulo; C.D. Seabra, Federal University of Sao Paulo / Marine Science; V.P. da Silva, Federal University of Sao Paulo / Biosciences; M. Dias, Sao Paulo State University / UNESP, H. Yamamuru, Catholic University of Santos; D. Ribeiro, Federal University of Sao Paulo / Biosciences

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 estuary 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: rats were exposed for five days to filtered water, 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 micromuclei frequency and DNA breakage in bone marrow and hepatocytes of rats exposed to estuarine water when compared to negative control (negative controls were not exposed to estuarine water). There were no histopathologic alterations and differences in the number of apoptotic bodies between the groups. K67 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
Molecular Toxicity of Metal Mixture and Defense Mechanisms in Zebrafish (Danio rerio)
S. Maidl, University of Antwerp, Dept. Biology; K. Smeets, University of Hasselt / Science; R. Blust, University of Antwerp Faculty of Sciences

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 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, however, raises interesting concerns with regard to oral exposure. Oral 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, bone, 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 hepatotoxins 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), Eif2 subunits G (Eif2G) and J (Eif2J), 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, Eif2G, Eif2J 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 initiates the endoplasmic reticulum stress 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)

W. Kim, Gwangju Institute of Science and Technology / School of Earth Sciences and Environmental Engineering; J. Park, S. Kim, Gwangju Institute of Science and Technology.

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, it is the model species of a new OECD test guideline (Test No 243: Lumyena stagnalis Reproduction Test). However, a reference genome was still lacking for this species. This situation motivated us to set up a multidisciplinary consortium, “lumyena STAGnals INternational Genome 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 self-mating. Whole genome shotgun sequencing of short fragments was performed with HiSeq2500 (2x250bp) and MiSeq (2x300bp) technologies (fragment size 400-700 bp). Scaffolding was based on the sequencing of mate-pairs 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 mapping 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, RNAsq data, protein mapping). Transcriptomic data obtained from Illumina and Nanopore technologies were combined to produce long and accurate synthetic reads. Genomic mapping of Illumina reads was performed. A total of 22499 genes (3492 without intron), with a mean/median length of 15299/8502 bp, and a mean/median GC content of 51.3%/57.5% and 75.5/5. 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 Lumyena 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).

**WE015**

Reference genome of the freshwater snail Lumyena stagnalis, a multidisciplinary invertebrate model

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The great pond snail *Lumyena 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: Lumyena stagnalis Reproduction Test). However, a reference genome was still lacking for this species. This situation motivated us to set up a multidisciplinary consortium, “lumyena STAGnals INternational Genome initiative” (STAGING). Taking advantage of the ability of this simultaneous hermaphrodite to self-fertilizing, we sequenced a highly homozygous genotype obtained through several generations of alternating selfing and self-mating. Whole genome shotgun sequencing of short fragments was performed with HiSeq2500 (2x250bp) and MiSeq (2x300bp) technologies (fragment size 400-700 bp). Scaffolding was based on the sequencing of mate-pairs 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 mapping 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, RNAsq data, protein mapping). Transcriptomic data obtained from Illumina and Nanopore technologies were combined to produce long and accurate synthetic reads. Genomic mapping of Illumina reads was performed. A total of 22499 genes (3492 without intron), with a mean/median length of 15299/8502 bp, and a mean/median GC content of 51.3%/57.5% and 75.5/5. 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

The environmental endocrine compromise 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

T. Procházková, Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University / Research centre for toxic compounds in the environment; J. Vecerková, B. Jarosova, Masaryk University / Research centre for toxic compounds in the environment; D. Trigo, I. Verdú, D. Matematica y de Fluidos Zoología y Antropología Física; J. Martinez-Guitarte, M. Novo, UNED / Fisica.

This study evaluated the effects of bisphenol A (BPA) and Bis(2-ethylhexyl) phthalate (DEHP), and test species were soil nematode *Dendrobaena* and *Folsomia candida* (*WE017*) and *Caenorhabditis elegans* (*WE018*). From the concentrations and effects, NOECDEHP.Rep and EC10DEHP.Rep of 500 mg/kg dry soil and 12.2 mg/kg dry soil. This study can be a source for biomarkers since several families of cytochrome P450 and GSTs might be involved in the detection of biological effects by the exposure of earthworms to DEHP. Effects of endocrine disrupting chemicals  (EDCs) to earthworm *Eisenia fetida* were investigated in the 2015 SETAC Europe 27th Annual Meeting Abstract Book.
toxic compounds in the environment; E. Szychra, Masaryk University; A. Skodova, M. Pernica, Z. Simek, J. Kohoutek, Masaryk University / Research centre for toxic compounds in the environment; K. Hilscherova, Masaryk University, Faculty of Science, RECENTOX / Faculty of Science, RECENTOX

Compounds with estrogenic activity have been widely studied as they can have acrogenic effects. NOECs were established for C. elegans. F. candida are < 10 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 freshwater. 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 aquatic environment. During 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 HE-Ro-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 they were not included in the calculation 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 estrogenic activity of water samples. However, the estrogenic equivalent of many samples was higher than could be anticipated from the biological analysis 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. Kin, J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Health Science

This study investigated the adverse effect of endocrine disrupting chemicals (EDCs) on soil organisms. Test EDCs include Bisphenol A (BPA) and Bis(2-ethylhexyl) phthalate (DEHP), and test species were soil nematode (Caenorhabditis elegans) and Folsomia candida) that are familiar standard test species for soil eco-toxicity test in ISO and OECD guidelines. The survival and reproduction assays for soil nematodes were conducted following OECD guideline. Ten F. candida were exposed to BPA and DEHP amended LFA 2.2 soil, and survival and offspring numbers were observed. For soil nematode population toxicity test, we observed offsprings of C. elegans. In the result, NOEC_{BPA} (BPA, reproduction) and EC_{10}_{BPA} of F. candida were 500 mg/kg dry soil and 191.4 µg/mg dry soil. NOEC_{BPA} (BPA, reproduction) and EC_{10}_{BPA} of C. elegans were 500 mg/kg dry soil and 2.4 mg/kg dry soil. On the other hand, LC_{50}_{BPA}, LC_{50}_{BPA} of F. candida were 2655.2 and 2275 mg/kg dry soil respectively. In the soil population of soil nematodes, NOEC_{BPA} (BPA, reproduction) and EC_{10}_{BPA} of C. elegans were 100 mg/kg dry soil and 36.7 mg/kg dry soil. NOEC_{BPA} (BPA, reproduction) and EC_{10}_{BPA} 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. in

WE019
Endocrine disruptors in soil: effects of Bisphenol A on growth, reproduction, immunity and gene expression in earthworms
I. Verdú, D. Trigo, Universidad Complutense de Madrid / Soil Zoology Group, D. Ziegler, A. Antón, Universidad de Vigo / Fisiac; J. Martínez-Guitarte, M. Novo-UNESE / Física Matemática y de Fluidos

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 10000 mg/kg dry soil in 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 no direct sensitivity in both species. By using the filter paper contact test (OECD, 1984), the potential estrogenic effect directed 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, AdiopR), detoxification (GST, Mi), stress (Hsc70, SOD), epigenetics (DNMT1, DNMT3b, Hist2) and genotoxicity (PARP1). Research funded by the Spanish Government. Projects: CMT2015-64913-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. Gonzalez, UNED / Mathematical Physics and Fluids; J. Martinez-Guitarte, UNED / Física Matemática 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-dimethylaminobenzozate (OD-PABA) showing that fourth instar larvae are affected by OD-PABA increasing the mRNA levels of chenodeoxycholic acid 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, cyp13a1, 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), CMT2015-64913-R.

WE021
Effects of endocrine disrupting chemicals (EDCs) to earthworm Eisenia fetida
R. Cui, Konkuk University / Department of Environmental Sciences; J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Sciences

This study evaluated the effects of bisphenol A (BPA) and bis(2-ethylhexyl)phthalate (DEHP) to earthworm. Test earthworm was Eisenia fetida. No significant change in behavior or survival were observed 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 examined 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 environmental 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)
L. Bergsson, University of Gothenburg / Department of Biological and Environmental Sciences; J. Sturve, Goteborg University / BIOENV

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 fish from eutrophic airfields since the 1990s. 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 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.

Effects of Mercury and Pesticides on Thyroid function in juvenile fish

D. van Zomeren, RMIT University / School of Applied Sciences; L. Toogood, G. Kibrìa, RMIT University / School of Science
Numerous environmental stressors exert acute or chronic effects on the fish thyroid cascade. Such effects could be mediated via thyroid alterations, imbalance of plasma T4 and T3 levels or damage to the structure of the thyroid tissues (thyroid hyperplasia, hypothyroidism). The thyroid 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 in the case of androgens, male 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 hypothalamic-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 high concentrations used in the experiments. The study also reviewed comparative studies on the effects of mercury and other pesticides on thyroid hormones and development of fish species worldwide.

Commercial Fish Feed can contain Steroids that are Endocrine Disruptors

C.H. Soares, Universidade Federal de Santa Catarina / Biochemistry Department; I. Baptista, Universidade Federal de Santa Catarina; j.O. Querino, Universidade Federal de Santa Catarina / Biochemistry
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 steroids. 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 steroids such as β-sitosterol, stigmasterol and cholesterol in the ppm range. These steroids 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, assessing the physiological effects of xenobiotics, particularly those that are endocrine disrupters.

Toxicological Evaluation of Abietic acid in Tilapia

Orochomis 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 Picea. 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 study was to evaluate the environmental stressors that are associated with the remobilization of perfluorooctane sulfonate (PFOS) and perfluorohexane sulfonate (PFHxS) through bioaccumulation effect. The objective of this study was to evaluate the composition of several commercial fish feed traded internationally, in particular the presence of steroids. 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 steroids such as β-sitosterol, stigmasterol and cholesterol in the ppm range. These steroids 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, assessing the physiological effects of xenobiotics, particularly those that are endocrine disrupters.

Flooding - an inter-disciplinary 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), poly cyclic 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 anemular flow. 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 also flowover 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 least estimation screen (LES). Estrogen equivalent concentrations (EEQs) already determined in sediments from five sites from the River Luppe using the YES ranked 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 disruptors (EDCs) from the sediment will be evaluated. Finally, 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
H. Jeong, J. Kim, KIST Europe / Environmental Safety Group
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 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 TOXICOLOGY: UNFOLDING BEHAVIOURAL RESPONSES TO AIR ENDOCRINE AND REGULATORY TOXICITY (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.
C. Barata, CSIC / Environmental Chemistry; D. Raldúa, IDAEA-CSIC
One of the major challenges that faces today tissue/tissue-like assays 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 enabled 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 photometer 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 software and the DanioVision Observation Chamber (Noluds). Changes in speed, length travelled and preferred area (periphery vs center) were analysed and compared under several cycle of bright and dark background light. Obtained results demonstrated that the 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, venlafaxine) 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 nonmaterial ecotoxicity.
T. Botha, North-West University / School of Biological Science; V. Wepener, North-West University - School of Biological Sciences / Unit for Environmental Sciences and Management
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 genetic models. The advantage of a rapid reproduction cycle permits zebrafish bioassays to be studied all the way from embryo level up to 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 toxicology testing. Several endpoints including histological and anatomical similarities between the endothelial cells, blood brain barrier, social interactions and immunomodulatory 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 behavioural room kept at 25°C and individually placed into 1.1L Tecniplast tanks in front of a frontal camera setup. Fish were exposed to 1, 5, 10, and 100 µg/L of cadmium tellurium quantum dots with three functional groups (COOH, PEG and NH2) 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 areas within the system were marked: upper and lower tank as well as each area to determine where fish spent most of their time, areas 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 (Diasema zernyi) from a glacier-fed stream
T. Pescatore, University of Milano Bicocca / Dept. of Earth and Environmental Sciences; F. Bellamoli, MUSE-Museo delle Scienze Trento / Dept. of Invertebrate Zoology and Hydrobiology; V. Di Nice, Univ. Milano Bicocca; F. Miani, MUSE-Museo delle Scienze Trento / Dept. of Invertebrate Zoology and Hydrobiology; S. Villa, University of Milano-Bicocca / Department of Earth Environmental Sciences; V. Lencioni, MUSE-Museo delle Scienze Trento / Dept. of Invertebrate Zoology and Hydrobiology.
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 ecotoxicological 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 (Diasema zernyi) after the exposure (24, 48, 72 hrs) to several pesticides (chlorpyrifos, S-metholaclor, bosalcid, captan) and pharmaceuticals (ibuprofen, furosemide and trimethoprim). 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-metachlor and trimethoprim after 72hrs whereas the remaining pesticides decrease 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
S.A. Kohler, University of Portsmouth / Biological Science; A. Ford, University of Portsmouth / Biological Sciences; M. Parker, University of Portsmouth / Biological Sciences.
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 medications can also alter behaviour. The aim of this study was to determine neurobehavioural and ecotoxicological effects of antidepressant drugs after chronic and acute exposure. Thirty adult female Daphnia magna were exposed to environmentally relevant concentrations of fluoxetine and sertraline for 48 hours. The following endpoints were measured: locomotion, feeding and olfactory induction. Fluoxetine exposure led to a decrease in motor activity and less feeding when compared to vehicle controls but, there was no significant difference in olfactory induction between treatments. Sertraline exposure caused a decrease in motor activity and the time spent feeding, but no change in olfactory induction. Comparison of these endpoints with the known effects of antidepressants in humans suggest that these behavioural changes are not specific to daphnids and are not likely to be of relevance to crustaceans in the environment.

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concentrations of urban pollutants affects worms burrowing capacities and upper layer of polluted sediments where they could select the less polluted fine pollutants, which are finally accumulated in the sediment compartment of aquatic worm Limnodrilus hoffmeisteri is abundant in this extreme environment species are able to maintain populations in such biotopes, which implies specific takes advantage for wildlife, especially for foraging birds. However, what type of species). We showed that isopods are effectively able to choose between two 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 environmentally feasible screening method for the safety of chars for soil environment.

WE036 Behavioral responses to urban pollutants in a key bioturbator species, the oligochaeta Limnodrilus hoffmeisteri: P. Mathilde, M. Florian, UMR 5023 LEHNA, University of Lyon 1; M. Eric, A. Jérôme, UMR 5510 MATEIS, INSA Lyon; R. Damien, H. Frédéric, V. Laurence, UMR 5023 LEHNA, University of Lyon 1 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 function (and groundwaters quality). To understand 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 13um resolution). We hypothesized that sublethal concentrations of urban pollutants affect 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 also expected to show how variations in the burrowing behavior displayed by worms to find an unoccupied 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: C. Kim, H. Lee, University of Seoul / School of Environmental Engineering; N. Hwang, S. Bae, Kangnam University / Department of Biology; S. Park, Kangnam University / Department of Environmental Engineering; H. Kim, S. Joo, Kangnam University / Department of Biology; J. Lim, Kangnam University / Department of Biology. 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 isopetbrates are an important part of soil fauna, having major roles in decomposition of organic matter and mixing of the soil, thus ensuring the homogeneity and fertility of the soil. To evaluate the impact of these materials on the behavior of Porcellio scaber we exposed them to different light-dark cycles of 15 minutes and duration of 24h. Functional implications of this burden is not yet fully assessable. The environmental impacts of isopods on the soil microorganisms and 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 fluxoxetine in birds: K. Arnold, S. Whillock, University of York / Environment; K. Herborn, Newcastle University / Centre for Behaviour and Evolution; J. Lane, Animal and Plant Health Agency / Department of Environment, Food & Rural Affairs; M.G. Pereira, Centre for Ecology & Hydrology / Lancaster; R. Shore, Centre for Ecology & Hydrology (NERC). Whether or not animals respond appropriately to novel and/or stressful stimuli will directly impact upon survival. The antidepressant fluxoxetine (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 fluxoxetine at concentrations reflecting exposure via feeding on invertebrates from wastewater treatment plants, showed alterations to their behaviour or physiology. Fluxoxetine-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 vasocostriction, potentially provides a relatively quick and easy method of assessing contaminant-induced stress in homeotherms. Moreover, key behaviours, for which there are established assay protocols in toxicology 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: M.E. Nielsen, L.F. Marcussen, P. Rosley, Aalborg University / Biology and Environmental Science. 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, fluxoxetine, and citolopram. Changes in behavioral patterns in 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 moultng. Changes in swimming behavior of D. magna was quantified by video tracking of single organisms followed by data analysis. 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). The challenges associated with for evaluating sublethal effects of pharmaceuticals to non-target organisms.
Chlorpyrifos effects at different levels of ecological hierarchy: link between changes in biomarkers (sub-organism level) and behavioral changes in Daphnia magna (organism level)

P. Caffiero, University of Milano-Bicocca; A. Finizio, University Milano - Bicocca / Department of Environmental and Landscape Sciences and Earth Sciences; M. Parolini, University of Milan / Biology

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 Regulations (ERA) in the European Union. The potential effects of pollutants on 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 organisms, 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.

Assessing the Ecotoxicological Potential of Carbamazepine - Evaluation of a Behavioral 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-well flat-bottom-plates and exposed to carbamazepine. The embryos were incubated at 28.5 ± 0.5°C. Effects were quantified by counting the number of pre-trial movements per second during 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.

Behavioral responses of marine vertebrate (Solea senegalensis) early life stages to water exposure effects of Carbendazim and 4MBC

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Solea senegalensis (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 monitoring 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 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 oceans 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 radiates 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.

The critical window of exposure of mixture toxicity of benzene, tolune 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 potentials, 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 dmnt, cat, vgt1, cyp1a, gsp, sod2, erb, serp1 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 alteration 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 (altering behavior) alterations. In addition, we showed that very early exposures could be utilized for better risk assessment of the respective chemicals. Keywords: Zebrafish, Behavior, Mixture toxicity, Benzene, Toluene, Formaldehyde
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 MBF. 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 MBF *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 aquaculture in media and for real time monitoring of the water quality degradation due to seawage 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 was compared with two freshwater amphipods, *G. fossarum* and *N. aquiles/casparyi as well as the isoped P. slavus in their sensitivity towards acute Cu-pulses (24 h) and their resilience after 7 days of recovery. Seven concentration levels between 5 and 720 µg/l were tested using the Multispecies Freshwater Biomonitor (MBF) to record quantitatively and continuously the individual survival and behaviour (locomotion). Locomotion decreased in a concentration-dependent manner in both *N. casparyi* and *P. slavus*, 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 hatching and continued on for 7 weeks. After 7 months of remediation in clean water three non-reproductive behavioral tests (novel tank, shoveling 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 aquarium 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 *Nauphoeta cinerea* exposed to BPA. Adult *P. reticulata* and *D. pygmaeus* showed an increased 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 MBF 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 MBF again. The MBF 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 (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 landing behaviour of the three aphid species on host plants could be influenced by compounds from the 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 the test 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 the test conditions. Owing to the fact that these tests can not distinguish between deterrent and attractant effects, 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.

**WE050**

Metaecoding of eDNA from Suspended Particulate Matter (SPM) for fish monitoring


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 sentinel DNA barcoding 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 multispecies and imaging analysis to predict the presence of individuals. 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 <15°C. We assumed 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 Cyb, 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 obtained populations and that are likely to affect fitness and on sex ratio towards during development in the three-spined stickleback after a long period of increased number of crossings to the black compartment in the scototaxis test. In the water three non-reproductive behavioral tests (novel tank, shoaling and scototaxis) tested sewage effluents. Recent studies have shown persistent effects on the behavior of Porsch-Hällström, Södertörn University / Dep of Nat Sciences Environment and Sciences, Technology and Environmental Studies; M. Kellner, Södertörn WE046 stress level in Multispecies Freshwater Biomonitor (MFB) to record quantitatively and Seven concentration levels between 5 and 720 ug/l were tested using the sensitivity towards acute Cu-pulses (24 h) and their resilience after 7 d of recovery. G. fossarum and N. aquilex/casparyi as well as the isopod P. slavus in their and drinking water monitoring we compared adults of two freshwater amphipods, and feeding were studied. Regarding acute toxicity (survival and locomotion) responses: initially in stream water, followed by 24 h of BPA-exposure. Thereafter, 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 and feeding were studied. Regarding acute toxicity (survival and locomotion) chronic expires (4 weeks) revealed significant effects on behavior at 0,1 mg/l BPA (Bisphenol-A) is being used worldwide in plastic products and threatens the by observing their behaviour in the control water and by comparing the treated to the control group adult fish were kept in four test conditions. T-maze olfactometer was used to investigate the effects of the essential compounds of essential oils are considered to be alternative controlling means of W. pyri and M. dirhodum. This study was addressed? (P). Compounds of essential oils are considered to be alternative controlling means of W. pyri and M. dirhodum. 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miRNAs) and; 2) the viral growth kinetics and replication. Chicken fibroblasts will
1) virulence of GaHV-2 infection (by modulation of expression of pathogenic
complex interaction between host and GaHV-2 involves viral microRNAs
cause of the induction of Marek's disease. Pollutants are known to
alter the
of chicken fibroblasts). Here, we will describe the effect exposure to PCBs has on:
induces Marek's disease, a lymphosarcoma, in chickens,
extraordinarily complex, even when the toxicity values are
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
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
mean and variance of the different species are usually kept
forward laboratory-to-field extrapolation. Moreover, comparing species sensitivity
rate constants for damage accrual and effects can be predicted based on
key GUTS feature is that an additional mortality due to the stressor occurs when the
varies between individuals and when exceeded, the individual dies (individual
to an effect into account and describes mortality on the basis of internal
routes for damage accrual. GUTS proper allows for the set-up of different
and feeding inhibition.
For a more realistic risk assessment for chemicals, it is also of basic interest to test
monitored for the water louse
A.aquaticus
hence, this exercise shows that TK/TD model predictions can be used to predict
compounds were adding up. These observations could be captured by the TK/TD
increase in feeding inhibition. In the 1990s, the viral infection was associated with a polynuertis syndrome, with a low rate of mortality. Concomitant
with increasing industrialization and environmental pollution in the 1960s, the acute
case of the disease appeared, with significantly higher mortality rates. The
complex interaction between host and GaHV-2 involves viral microRNAs
(miRNAs), miRNA expression is measurable (miR-m) and can be followed in
various stages of infection 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-M has been shown to be the direct cause for the induction of the disease. Expression of miR-M is found in the infected
fibroblasts and not in uninfected control cells. This implies that these cells are not compatible with 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) viral miRNA expression (by measuring miRNA expression)
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). Viral 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)

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
Heparse 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, when an appropriate QSR is unavoidable. 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
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
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.

MOSAIC in support of statistical analyses from standard bioassay data
S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutionary Biology
MOSAIC stands for “Modelling and Statistical tools for ecotoxicology”. In
ecotoxicology, bioassays are standardly conducted in order to measure acute or
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,
managers, NGOs and other stakeholders. Without wasting time on
extensive mathematical and statistical technicalities, any user is given advanced and
intuitive methods for handling and interpreting ecotoxicological data. MOSAIC offers three operational tools: (i) MOSAICcap, 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 hazard
concentration for 50% of the species (HC50) or, even when the toxicity values are
censored; (ii) MOSAICsim, which provides users with a complete statistical
analysis of bioassay reproduction data simultaneously accounting for mortality all
along the bioassay. MOSAICcap uses various concentration-effect models within a
Bayesian framework. The model that best account for the observed variability is
selected and provides EC50 estimates; (iii) MOSAICsim, which provides users with a complete statistical analysis of bioassay survival data. A log-istic model is fitted
within a Bayesian framework to provide LC50 estimates. Based on a toy example,
this talk will illustrate how MOSAIC can help ecotoxicologists, regulators,
managers, NGOs and other stakeholders in statistically and 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 Evolutionary Biology; S. Preux, University Lyon / Laboratory of Biometry and Evolutionary Biology; V. Ducrot, Bayer CropScience AG / Environmental Safety Toxicology

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 (‘mose’ 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, DEBox 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. Gerss, 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

Aquatc 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. e.g. the extrapolation of unstated 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 more accurately analyse 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 models
A. Gerss, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change; K. Ladermann, gaiac - Research Institute for Ecosystem Analysis and Assessment; N. Lippmann, Research Institute gaiac; M. Hammers-Wirtz, Research Institute gaiac; S. Classen, gaiac Research Institute for Ecosystem Analysis and Assessment

Aquatic effect assessment uses results from short-term laboratory experiments performed under constant environmental conditions. In contrast, populations regularly experience fluctuating environmental conditions, such as seasonal or diurnal variability in ambient temperature, which hampers a straightforward laboratory-to-field extrapolation. Moreover, comparing species sensitivity might be biased by experimental conditions as different species are usually kept under different environmental conditions. In contrast, physiological models of organisms provide insights into the mechanisms how these different environmental conditions influence 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 physiological measures such as filtration rates or developmental rates. The suggested temperature extension to GUTS will allow the simulation of toxicity under more natural conditions and will facilitate the cross-species comparison 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, Altaerra Wageningen University and Research Centre / Environmental Risk Assessment Team; D. van den Brink, Altaerra Wageningen University / Analytical and Environmental Sciences; L. Boerwinkel, L. Buijse, J. Gough, J. Woestein, J. van den Brink, Altaerra and Wageningen University / Toxmod Research Institute; M. Hammers-Wirtz, Research Institute gaiac; S. Classen, gaiac Research Institute for Ecosystem Analysis and Assessment

Aquatc 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. e.g. the extrapolation of unstated 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 more accurately analyse toxicity data for various aquatic invertebrate species exposed to chlorpyrifos and to derive more general insights into the dynamics of mortality and feeding inhibition.

WE064
Modelling the toxicity of organic chemicals on aquatic invertebrates: maximizing applicability based on minimal data
A. Focks, Altaerra Wageningen University and Research Centre / Environmental Risk Assessment Team; P. van den Brink, Altaerra and Wageningen University / Toxmod Research Institute; M. Hammers-Wirtz, Research Institute gaiac; S. Classen, gaiac Research Institute for Ecosystem Analysis and Assessment

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 dipterus 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 Calperum 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.
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 published. For Ecotoxicologists, this is thinning out the capability for predicting 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 TOXSWSA. The software tool is a combination of non-standalone and command-line applications 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 reproducible for all involved parties to increase acceptance of such models to be commonly used as a higher tier option in ecotoxicological risk assessment, and as actually intended also by EFSA. It is obvious that one has to become familiar with all available tools for environmental impact assessment 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, besides the computer science, also has to have a deep knowledge in natural science and should best be familiar with the regulatory framework. A modelling tool in higher tier aquatic risk assessments can be the toxicokinetic and toxicodynamic General Unified Threshold model of Survival (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. Moreover, we implemented an additional functionality which link the outcome of GUTS with exposure files from the environmental fate tool TOXSWSA. The user can easily extract the exposure profiles from TOXSWSA 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
J. Becker, UFZ Center for Environmental Research / Department of System Ecotoxicology; T. Jager, DEBox Research / Dept of Theoretical Biology; S. Kranmer, Schiff, Leibnitz Institute for Zoo and Wildlife Research; S. Duquenne, UFZ Center for Environmental Research (UBA) / Plant Protection Products; S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products; M. Solé, German Federal Environment Agency UBA; J. Wogram, German Federal Environment Agency UBA / IV 2.4 - Ecotoxicological Assessment; M. Lies, UFZ Center for Environmental Research / Department of System Ecotoxicology
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-inocosm 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 (GACR, 2010) as well as in various SETAC workshops. Despite the uncontroverted 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, NORDFOKUS (2013) a set of 27 mechanistic population models have been very helpful and established for 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
T. Meiers, Bayer AG / Environmental Safety; T. Segen, Bayer AG / Environmental Modelling; S. Bub, Tier 3 Solutions; M. Wang, WSC Scientific GmbH / Dept E fate Modelling; G. Goerlitz, Bayer CropScience AG / Environmental Safety
Nature conservation, Building and Nuclear safety (BMUB), a critical evaluation of risk assessors to weight the results of a model analysis in comparison with other non-standard test species, e.g. while for Aquatic species in four Puget Sound Basin watersheds: The Lower Skagit River, Unibersity; K. von Stackelberg, Harvard School of Public Health / Environmental Research Institute for Ecosystem Analysis and Assessment; T. Preuss, Bayer AG / Environmental Safety
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. In 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, lumped 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 extracted from a set of supplier simplified or more genetic 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 model can also 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 gaia / gaia - 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 analysis and compare results of these laboratory tests. Ecophysiological processes such as growth and reproduction were simulated with the individual-based population model D4MP dependent on experimental conditions, whereas mortality was described by the TK-TD modelling framework GUTS. With these models, reproduction per tested animals 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
J. Koch, GhEnToxLab (Ghent University) / Environmental Toxicology Unit - GhEnToxLab; C. Jensen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; K. De Schamphelaere, Ghent University (U Gent) / Applied Ecology and Environmental Biology
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 mechanistic population models is an 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 stress 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 the long term and population level. 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, lumped 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 extracted from a set of supplier simplified or more genetic 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 model can also 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.
invertebrate model organisms in ecotoxicology. As most crustaceans, they undergo sexual reproduction and thus form a welcome alternative to the still more commonly used parthenogenetic 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 parameterize 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 these copepods. 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 antipredator citalotrop. The DEBkiss IBM was used to analyse this drug’s toxicological MoA on N. spinipes.

WE070 DEB(tox) models - Can we predict under different environmental conditions? B. Gousson; University of York / Environment Department; C. Rendal, E. Butler, Unilever / Safety and Environmental Assurance Centre SEAC; R. Ashauer, University of York / Environment; O. Price, Unilever / Safety and Environmental Assurance Centre SEAC

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 multi-stress 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 organism's 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 untested environmental conditions (temperature and feeding level) was analysed and discussed.

WE071 Population Models for Use in Bayesian Networks and Adverse Outcome Pathways J.D. Stark, Washington State University / Dept of Entomology; C.J. Mitchell, Washington State University / Puylaccur Research and Extension Center; W.G. Landis, Western Washington University / Institute of Environmental Toxicology; S.E. Graham, Western Washington University; M.E. Harris, Western Washington University; K. von Stackelberg, Harvard School of Public Health / Environmental Health

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 assessment. This work will be added to existing study to be used in the developed and documented in the scientific literature. In many cases the models have only to be assessed if this module were built together in a meaningful way. This will present our experience with this kind of modular approach.

WE072 Conceptual model for implementing community model output in Ecological Risk Assessments of chemicals A. Palmqvist, Roskilde University / Department of Science and Environment; T. Strauss, Research Institute gaia/ gaia - Research Institute for Ecosystem Analysis and Assessment; S. Claussen, gaia / Research Institute for Ecosystem Analysis and Assessment; M. Hammers-Wirzt, Research Institute gaia; A. Gergs, gaia / Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change

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. The current long-term 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 the community model in ERA (e.g. 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 K.H. Vlasminck, Ghent University; K. De Schamphelaere, Ghent University (UGent) / Applied Ecology and Environmental Biology; P. Van Sprang, ARCHE; K. Viane, Arche consulting

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, development (Lymnaea stagnalis) and reproduction (Lymnaea stagnalis) have been demonstrated, as well as effects on reproduction, development (Lymnaea stagnalis) and reproduction (Lymnaea stagnalis) have been demonstrated, as well as effects on reproduction, development (Lymnaea stagnalis) and reproduction (Lymnaea stagnalis) have been demonstrated, as well as effects on reproduction, development (Lymnaea stagnalis) and reproduction (Lymnaea stagnalis) have been demonstrated, as well as effects on reproduction, development (Lymnaea stagnalis) and reproduction (Lymnaea stagnalis). The model was parameterised on life cycle data from literature and additional test data, OECD 211; acute immobilization test, OECD 202) are standard tools in the population models (Lymnaea stagnalis). 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 indicated that desiccation 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 Y. Kim, Y. Lee, Korea University; J. Wee, Korea University / Division of Environmental Science and Ecological Engineering; K. Cho, Korea University / Division of Environmental Science and Ecological Engineering

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.
Understanding the uptake dynamics of Daphnia is important to consider 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 temperatures (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 IEM

**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 solvents (e.g. dimethylformamide, dimethyl sulfoxide) at 0.1 mL/L (100 mg/L) or less. Even though 0.1 mL/L solvent is not toxic to test organisms, 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, Adolfson-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 T2G21, 202, 203, 210, 211).

Especially, practical problems of passive dosing during long-term test using flow-through exposure systems were reported. 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 ecosystem studies
M. Castro, Stockholm University / ACES; B. Yuan, J. Athanassiadis, Stockholm University / Department of Applied Environmental Science, and Analytical Chemistry ACES; M. Breitholtz, Stockholm University / Department of Environmental Science and Analytical Chemistry

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 contaminants because 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 PBTE (i.e., persistent, bioaccumulative and toxic) and TCPB classed (i.e., very persistent, very bioaccumulative) substances. However, medium (MCCPs) and long chain (LCPPs) 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 (SCCPs, MCCP, LCCP, 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 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 test 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
D. Schaefer, Bayer CropScience / Environmental Safety; L.L. Lagadic, Bayer AG, Crop Science Division / Environmental Safety; M. McCooe, Bayer CropScience / Environmental Toxicology and Risk Assessment; P. Radix, Bayer AG Crop Science Division / Environmental Safety; T. Xu, Bayer AG Crop Science Division 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 environmental 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
L.W. University of California, Riverside / Department of Environmental Science Synthetic pyrethroids are an important class of contemporary insecticides used in

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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 research 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 ($C_{DW}$) in surface water and sediment porewater, and correlation of $C_{DW}$ with 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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Persisting 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 fishes and its relationship with the surrounding land uses. Fish were collected in three different areas of Argentina: I. the Negro River basin (41º56’S-5º29’W). Cyprinus carpio, Odontesthes hatcheri, Oncorhyncus mykiss, and Perccidchys coloquiquitu; II. Dulce creek basin (37º5’S-57º15’W) Rhamdia quelen; and III. Coastal waters of Southwest Atlantic 34º41’S Callorhinichus callorynchus, Symphysrygia bonaparti, 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-ECID. 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. The sampling area was from the basin, such as soils, macrophytes and sediments. Catfish from Central-East region (5.5-17.2 ng/g ww) and chondrichthyns 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 chlorpyrifos and cypermethrin are used.

Despite the similar pattern, marine chondrichthyns 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 of our environment. This work was 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, the 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 retardant in the water of the US Great Lakes using polyethylene passive sampler C.A. McDonough, University of Rhode Island / Graduate School of Oceanography; P.A. Helin, Ontario Ministry of the Environment & Climate Change / Environmental Monitoring and Reporting Branch; D.C. Muir, Environment Canada / Aquatic Contaminants Research Division; R. Lohmann, University of Rhode Island / Graduate School of Oceanography

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 alkyl-PAHs. BDEs 15, 28, 47, 99, 100, 153, and 183 were found at >50% of locations. $\Sigma$BDE ranged from below detection limits at an offshore buoy in eastern Lake Erie to 41 pg/L near the shoreline of western Ontario in Toronto, Canada. Among the NHFRs, pentabromobenzene (PBB), pentabromobenzylbenzene (PBBE), hexabromobenzene (HBB), and analizable chlorinated plus (ADP) and SDP) hydrophobic compounds in the environment. The concentration ratio of passive samplers with different thicknesses. Passive sampling in kinetic mode: Determining dissolved concentrations by 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.

WE085 Sorption studies of polychlorinated biphenyls (PCBs) between aqueous solution and soil particle grain sizes: Effects of temperature and ionic strength. G.C. Adeyinka, B. Moodley, University of Kwazulu-Natal / Chemistry Introduction. The PCBs belong to the most persistent, bioaccumulative, and toxic group of contaminants that pose threats to both aquatic and terrestrial 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 (Eriason, 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 organic pollutants (PCBs) on the partitioning of selected water and soil contents between aqueous solution and various soil particle grain sizes. \textit{\textbf{<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 investigated 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. \textit{\textbf{<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 different soil particle grain sizes. The result of batch experiments showed that the sorption capacities for PCB congeners with different soil particle grain sizes. The smallest 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 a 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.

\textit{\textbf{WE086 Quantification of PCB and PCDD partitioning constants between water and dissolved phases by polymer depletion technique}}

V. J. Schacht, S. Grant, The University of Queensland, Queensland Alliance for Environmental Health Sciences; E. Escher, Helmholtz Centre for Environmental Research GmbH - UFZ / Cell Toxicology; D. Hawker, Griffith University / School of Environment; C. Gaus, The University of Queensland / Queensland Alliance for Environmental Health Sciences

Determination of the partitioning behaviour of highly hydrophobic contaminants (hHOCs, log Kow > 5.5), many of which can be highly toxic e.g. dibenz-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 feasible. 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 Kow 5.8-8.3) between selected water and soil contents. The linear relationship between log

\textit{\textbf{WE087 Prediction of the Formation of Biogenic Non-Extractable Residues}}

S. Trapp, DTU Environment / DTU Environment; A. Brock, DTU Environment; M. Kästner, Helmholtz centre for environmental research - UFZ / Department of Ecotoxicology

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 composition. 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 substrates that includes the Nernst equation and the availability of electrons to biological processes. Labelled substrate turns into metabolites, biomass, new extractable residues (NER) and CO₂. 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 the the decay rate default values can be used. Thus, only few parameters remain. The simulation results are contrasted to experimental studies. In such degradation experiments with labelled 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 CO₂ formation during their decay. This allows to calculate bioNER formation and consequently the amount of both. We have developed a new pathway-independent method.

WE090
Testing of viscous liquid mixtures D. Thomas, Smithers Vis cient 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 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 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 1x10⁻³ 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 M. Turk-Sekulic, University of Novi Sad / Department of Environmental Engineering and Occupational Safety and Health; S. Pap, University of Novi Sad Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; N. Boskovic, University of Novi Sad, Faculty of Technical Sciences; M. Milanovic, University of Novi Sad, Faculty of Medicine; J. Radonic, Faculty of Technical Sciences / Department of Environmental Engineering and Occupational Safety and Health; B. Obrovska, Faculty of Technical Sciences, Novi Sad / Department of Environmental Engineering; N. Grujic Letic, Pharmacy
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 thermalchemical conversion (H.PO₄ , 500 °C) in the complete absence of inert atmosphere. Obtain eco-friendly activated carbon (CsPcA) 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 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, qmax, 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. Prepared study showed that activated carbon prepared from cherry/sweet cherry kernels is useful adsorbers because it is cheap, recyclable and highly efficient. According to obtained results, the CsCpA was considered as a promising adsorbent for the removal of investigated pharmaceuticals 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 (HR-MS/MS) 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 Henrikslund municipal WWTP in Stockholm, Sweden in 2014. The samples were analysed with a multi-pollutant screening method called internal standards of a wide group of polar pollutants (mainly for quality control purposes). After filtration, the samples were analysed 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 different removal during 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 process. 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/Aerobic Digestion Treatment on Concentrations of Plasticizers in Wastewater Sludge D.L. Armstrong, University of Maryland College Park; R.N. Hartman, University of Maryland College Park / Department of Civil / Environmental Engineering; C.P. Rice, U.S. Department of Agriculture / Sustainable Agricultural Systems Laboratory; M. Ramirez, DC Water and Sewer Authority; A. Torrents, University of Maryland / Civil and Environmental Engineering
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 bioaccumulative 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 aerobic digestion (TH-AD). Concentrations of the target analytes in biosolids produced by the TH-AD process, sludge 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 E. Rudelj, Aalborg University / Department of Civil Engineering; A.H. Nielsen, Aalborg University / Department of Civil Engineering; U. Bollmann, K. Bester, Aarhus University / Environmental Science; J. Vollertsen, Aalborg University / Civil Engineering
Biocides are present in many building materials to protect them against microbial growth. One such material is render where silicone or acrylic 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, it is still little known 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 isoprotron, octyl-isoctazolinone, diuron, terbutryn, cybutrin (irgarol 1051), dichlorocontrol-isoctazolinone, isocarbox, 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 extractive experiments with a continuous wet render showed that 4 of 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, Thiacloprid, Clothianidin, Dinotefuran, Thiamethoxam and Nitenpyram are familiar. 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 are very 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 pollutant in the full, to accumulate them on to absorbent 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 neonicotinoid insecticide standard kit has been established to combine two techniques. “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

K. Knudsmark, University of Copenhagen / Department of Environmental Science

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 wastewater treatment plants and has been used as fertilizer. It 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 (Ci) at equilibrium with the sludge and iii) convert the concentrations into freely dissolved concentrations (Ci) 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 and extracted in four phases with 10, 100, 1000 and 10000 μL of 10% ethanol. From the 10, 1000 and 10000 μL extract, 4 mL ethyl acetate was analysed for 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 (Ci) was obtained. The chemical activity ratio (AR) was then determined as ratio of Ci to Ci. The PAHs before and after digestion were determined. The freely dissolved concentrations (Ci) were calculated using silicone-water distribution coefficients (Ks) 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 on its site accumulation during their deployment. But passive sampling techniques have also disadvantages to measuring micropollutant in particulate phase. E.g. calibration of the passive sampler and 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. Grab sampling is a low flow rate sampling mode of water depending on water turbidity. As a 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 muddly surface water for a week. In this study, a novel autonomous sampler “GRAVE” were developed and applied to surface water monitoring. “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 OPES

L.O. Okeme, University of Toronto / Physical and Environmental Sciences; L.M. Jantunen, Environment Canada / Centre for Atmospheric Research Experiments; M.L. Diamond, University of Toronto / Department of Earth Sciences

Semi-volatile compounds (SVCs) 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 in the gas-particle phase. Using gas-particle fractionation of OPES (OPES), as SVCs, 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^-10^-10 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 (Sihling 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 gas phase and the film of water covering the particle phase. 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 our time, and the concern micropollutants - substances that appear in concentrations ranging from 10^-10^-10 mPa to 10^-10^-10 mPa. Reference 61: SETAC Europe 27th Annual Meeting Abstract Book

WE104 Oxidative capacity of manganese peroxidase (MnP) towards micropollutants as potential biocatalytic treatment

T. Knudsmark, University of Copenhagen / Department of Environmental Science

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 our time, and the concern micropollutants - substances that appear in concentrations ranging from 10^-10^-10 mPa to 10^-10^-10 mPa. Reference 61: SETAC Europe 27th Annual Meeting Abstract Book

WE103 Evaluation of the potential of manganese peroxidase (MnP) in the biodegradation of recalcitrant hydrocarbons

K. Knudsmark, University of Copenhagen / Department of Environmental Science

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 our time, and the concern micropollutants - substances that appear in concentrations ranging from 10^-10^-10 mPa to 10^-10^-10 mPa. Reference 61: SETAC Europe 27th Annual Meeting Abstract Book
value of 10 m³. For values of 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 m³ 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.

**WE102 Study of the Degradation of Bisphenol A (BPA) by bisadductoisymes via HPLC in urban reservoir waters**

Q.C. de Lima, C. da Silva, C.C. Rodrigues, UFABC / CNCH; L. Nunes, D. Rosa, UFABC / CECs; V.V. Vitali, Instituto de Botânica / Micologia Aplicada

Contaminated water of one of the rivers in the metropolitan area of São Paulo that is managed by Empresa de Águas de São Paulo (EPA) and serves urban areas in central São Paulo was found to contain bisphenol A (BPA). This contaminant is currently considered a potential human carcinogen and endocrine disruptor. The study was conducted to determine the degradation of BPA by bisadductoisymes using HPLC with diode array detection (DAD) and ESI-MS/MS. The results showed that BPA was degraded to several products, including monoadducts and bisadducts. The degradation products were identified by comparison with standard compounds and by tandem mass spectrometry (MS/MS). The study also investigated the effects of different environmental factors, such as pH, temperature, and dissolved oxygen, on BPA degradation. The results indicated that BPA degradation was significantly influenced by these factors, with optimal degradation rates observed at pH 7, 25°C, and 10 ppm dissolved oxygen. The study highlights the importance of understanding the degradation of BPA in urban reservoir waters to mitigate the potential health risks associated with exposure to this contaminant.
pre-treatment of Diesel contaminated soil. This study also developed oil-degrading microbes (K-6) from C15-C22 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-2015R1D1A1A01059064)).

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

WE105 SETAC Chemistry Interest Group A.J. Jones, DuPont Crop Protection / Institute of Environmental Toxicology

Biochar amendment in dimethoate contaminated soils: toxicity assessment using the collembolan Folsomia candida and the dicotyledonous plant Enchytraeus albidus

P.M. Mouoliang, University of the Free State / Zoology and Entomology; P. Voua Otomo, University of the Free State / Department of Zoology and Entomology; E. Breedenhau, 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 organicisation 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. Therefore, 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 LC50 could not be calculated but a LC50 (47.90% sludge) was calculated. Consequently, an EC50 = 58.53% sludge was calculated for reproduction. For Harrismith, similarly, significant effects were only found on reproduction in the 75% per 100% sludge treatment (p<0.01). An LC50 could not be calculated but an LC50 (56.25% sludge) was calculated. The EC50 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 oligochaetes.

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 dimehotate 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 dimehotate 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 dimehotate 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 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) equilibrium 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-transportation processes 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 (MaxPEC,a) 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 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.

WE110b 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 11266-3 (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 used to demonstrate different statistical approaches (parametric versus non-parametric 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. Reference(s) De Jong, FMW., Van Beelen,
WE111
Stratified activity based sampling of soil micro-arthropods
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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 toxica nts 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 species it is imperative to determine what is crucial for the species survival. During the stratified 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 the soil was inoculated with 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. Sphaeriopsis pumilis (Sminthuridae) 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 Lepidopus cavanil) 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 variation. 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)
S. Pierer, German Federal Environment Agency (UBA); Plant Protection Products Programme; P. Craig, Durham University / Department of Mathematical Sciences; K. Dinh, DTU (Technical University of Denmark) / Section for Marine Ecology and Oceanography, DTU Aqua; M. Klein, Fraunhofer IME; R. Laskowskij, Jagiellonian University / Ecologyotoxicology Stress Ecology Group; B. Manachini, University of Palermo; R. Smith, School of Applied Sciences, University of Huddersfield; J.F. Sousa, University of Coimbra / Department of Life Sciences of University of Coimbra; I. Sundh, Swedish University of Agricultural Sciences; K. Swarowsky, German Federal Environment Agency UBA; A. Tiktak, PBL Netherlands Environmental Assessment Agency / Water Agriculture and Food; C.J. Topping, Aarhus University; M. Arena, EFSA - European Food Safety Authority / Pesticides 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 account the entries of the year 2007/2008 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 by the PPR Panel in an In-Soil 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 exposure section from the baseline studies to the risk assessment for specific key 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 PPPs on in-soil organisms, addresses possible direct and indirect effects of intended PPP uses. The second component assesses 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 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 in the SSD. The SSD approach was 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://www.efsa.europa.eu/en/topics/11750). Some of these metalos 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 in the HCS 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 HCS or HCS0 values (i.e., smaller confidence intervals), thereby increasing the robustness of the SSD. This poster will present the learning experience with metal SSDs for soil organisms.

WE114
Effects of Bacillus cereus inoculum on the ecotoxicity of metal-based fungicides towards Eisenia andrei in spik ed 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 citellate 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 tested, E. andrei showed significant (p< 0.05) increase in 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, earthworms 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 battery of plant protection products

J.F. Sousa, University of Coimbra / Department of Life Sciences of University of Coimbra; M. Arraiano, EFSA - European Food Safety Authority; A. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products; G. Mallmann, Universidade do Estado de Santa Catarina; O. Filho, Arbuscular mycorrhizal fungi (AMF) can establish symbiosis with 80% of different plant families contributing to crop productivity and ecosystem sustainability. Thus, placing them in their risk assessment test battery is not only important in maintaining stable soil biogeochemical cycles, but also 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 latter 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 extraradical mycelium, their ability to render surfaces hydrophobic and the production of gels and exudates, they 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 ecosystems, 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 Funnelliformis mosseae (formerly Glomus mosseae) is already available. However, further adaptation of the endpoints measured or its extension to other species/stains 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 asymptotic 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 worked presentation 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. Rijks, MTM Research Centre, Otrebro University / Ecosystem Ecology; A. Ekblad, MTM Research centre Otrebro University / Ecosystem Ecology. Ecological risks are currently identified to provide their vicarious ecological 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 CO2 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 13C-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 require the added sugar at similar rates in both polluted and unpolluted 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 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 significance of the site is crucial to determine whether remedial actions are needed to be taken.

**WE117**

Impact of pesticide on non-target fungi in soils and sediments

E. Oehl, Agroscope Wädenswil / Ecotoxicology; A. Aldrich, Research Station Agroscope ACW / Ecotoxicology; O. Daniel, Agroscope / Institute for Plant Production Sciences IPS

In this study, we refer to ‘non-target fungus’ (NTF) in soils and sediments and focus on the arbuscular mycorrhizal fungi (AMF). Our aim is to present the AMF to the 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. Sinsch U. & Leinweber G. 1984), and to highlight their potential roles in the rhizosphere of virtually all terrestrial and aquatic ecosystems colonised by vascular plants and fulfill 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 detectable hyphal mycelia and intraradical vesicles and arbuscules, 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 up a functional testing on the AM fungus Funnelliformis mosseae (synonym: Glomus mosseae) referring to ISO 10832:2009. In this protocol also an AMF hyphal root colonisation test was proposed, but the ‘hypal 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 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) EPPO Production of draft scientific opinion: Arbuscular mycorrhizal fungi for in-soil organisms, accession number 2016/0020/EPPO. EFSA PPR Panel (2018) Scientific Opinion on pesticide risk assessment for non-target microorganisms in terrestrial ecosystems: Impact of selected pesticides on the AMF F. candida and imidacloprid to earthworm (Eisenia fetida). EFSA J. 17:271. EFSA PPR Panel (2019) Scientific Opinion on pesticide risk assessment for non-target microorganisms in terrestrial ecosystems: Impact of selected pesticides on the AMF F. candida.
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 for spiked natural LUFAs (4,5,6 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 0.223 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 EC10 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 F. fimetaria. These results are in line with many recent studies questioning the use of neonicotinoids in agriculture.

WE121 Toxicity of Neonicotinoids Towards Two Soil Invertebrates C. Lima, Vrije Universiteit Amsterdam / Animal Ecology; C. van Gestel, VU University Amsterdam / Ecological Science
The present study aimed to answer three questions: (i) Does the toxicity of three different neonicotinoids (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 Lafa 2.2 soil. Thiametoxam (pure compound and formulation Actara®) was the most toxic neonicotinoid for survival of F. candida (LC50 0.36 and 0.4 mg/kg dry soil, respectively), while imidacloprid and thiametoxam had a similar toxicity for reproduction of this springtail (EC50 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 (LC50 0.80 and 0.74 mg/kg dry soil and EC50 0.40 and 0.50 mg/kg dry soil, respectively), while thiametoxam, both pure and formulation, did not affect earthworm survival and reproduction (concentrations tested up to 10 mg/kg dry soil). Thiacloprid was the least toxic neonicotinoid for F. candida and E. andrei with LC50 4.2-5.2 and 9.3 mg/kg dry soil and EC50 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 distribution 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 field 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?
A. Gainer, University of Saskatchewan - Toxicology Centre / Soil Science, Toxicology; R.A. Akre, University of Saskatchewan / Soil Science; S. Siciliano, University of Saskatchewan / Department of Soil Science
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 hydraulic lubricating oils (e.g., C14 petroleum hydrocarbons). 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 rate 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 C4 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?
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). 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 - 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. Furthermore studies discussing the present study will be presented.

WE124 Which are the key soil parameters driving the toxicity of chemicals to soil organisms?
P. Kotschik, Umweltbundesamt / Federal Agency of Environment / Risk assessment for plant protection products; J.F. Sousa, University of Coimbra / Department of Life Sciences of University of Coimbra; J. Roembke, ECT Oekotoxikologie GmbH; D. Felsmann, Umweltbundesamt / Federal Agency of Environment Germany; S.E. Egerer, Umweltbundesamt; S. Pieper, German Federal Environment Agency (UBA) / Plant Protection Products
According to current guidance, risks for soil organisms exposed to chemicals (e.g. plant protection products) are assessed in a first step by guided on standardised tests performed in artificial soils (e.g. [1,2,3]). These substrates are a mix of sand, peat, kaolinitic clay and carbonate minerals 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 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 unravelled 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 collemboles of three different PPP active substances in five different test systems and assessing their potential for modulating toxicity of different compounds to different organism groups. The selection of test systems is based on 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 describing the toxicity of chemicals to soil ecosystems. Besides this, the dataset will be included in a shortcoming analysis, helping to prioritize future research on this complex topic. [1] SANC0/10329/2002 (2002): Draft Guidance Document on Terrestrial Ecotoxicology. 17 October 2002. [2] OECD 222

WE125 Derivation of species sensitivity distribution for bisphenol A for the protection of freshwater ecosystems
J. Kwak, J. Moon, D. Kim, R. Cui, Konkuk University; Y. An, Konkuk University / Department of Environmental Health Science Bisphenol A is used for making plastics, copy pair, and recpet 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 protection 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 (RE20160353).

WE126 Terrestrial toxicity of silver nanoparticles using soil alga
S. Nam, J. Kwak, Konkuk University; Y. An, Konkuk University / Department of Environmental Health 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 motility, cell viability, 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 GAA project (20140005560001) 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
J. Weiss, Stockholm University, ACES / Department of Aquatic Sciences and Assessment; D. Berggren Kleja, A. Enell, Swedish Geotechnical Institute; M. Engwall, Orebro University / MTM research center Department of Science and Technology; M. Fransson, Swedish Transport Administration; S. Halin, C. Jones, Swedish University of Agricultural Sciences; M. Larsson, Orebro University / MTM research center Department of Science and Technology; L. Rosén, Chalmers University of Technology; A. Taylor, M. Viketof, Swedish University of Agricultural Sciences; Y. Volchk, Chalmers University of Technology; A. Aber, Swedish Geotechnical Institute; K. Wiberg, Swedish University of Agricultural Sciences / Department of Aquatic Sciences and Assessment. APPLICERA is a 4-year project aiming at developing a methodology 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 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 UAV survey in this area, a large 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 (2-4 PAHs, 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 soil-dwelling microorganisms, microbial activity 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 methodological approach. 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)
N.W. Nyvka, University of the Free State / Zoology and Entomology; P. Vouo Otomo, University of the Free State / Department of Zoology and Entomology Enchytraeus (Oligochaeta) are important soil promotors and provides various beneftis to plants. Once introduced, 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<0.05). Imidacloprid caused a significant decrease in the reproduction of E. albidus in both soils. However, a higher EC50 (46.23 mg/kg) in the biochar amended soil than in the non-amended one (EC50 = 22.77 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<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 enchytraeidae. 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. Wagelman, Bioclear earth

WE130 The gut wall of the earthworm Eisenia fetida harbours a closely associated bacterial community distinct to the soil community
E. Swart, Centre for Ecology & Hydrology; L. Newbold, H. Gweon, NERC Centre for Ecology & Hydrology; P. Kille, Cardiff University; D. Spurgeon, Centre for Ecology & Hydrology; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology The microbiome can play an essential role in the health 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 provide a unique perspective of the harmful impacts on the 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 sequencing of the bacterial community was assessed in culture soil, cast, non-depated guts, depurated guts and depurated guts that were washed with different intensities. Multivariate analysis showed that the community in the soil and cast and the non-depured 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,
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
M. Jang, Korea Institute of Ocean Science and Technology / oil and POPs research group OPRG; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; G. Han, Korea Institute of Ocean Science and Technology KGAD; Y. Cho, Korean Ocean Research and Development Institute of Ocean Science and Technology KORDI / Oil and POPs Research Group OPRG; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group

There are increasing observations of microplastic ingestion by marine organisms representing various trophic levels 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 programs by virtue of their broad geographical distribution, easy assessiblility, high tolerance to salinity, and limited mobility. Similarly, bivalves can be useful sentinels for the assessment of microplastic pollution in water column because they filter large volumes of seawater, while lugworms can reflect microplastic pollution in sediment bottom. In order to assess the contamination status of microplastics in marine invertebrates and their contamination characteristics according to different feeding habitats and regional sources, mussels (M. edulis), oysters (C. gigas), and lugworms (Perinereis albithuiltes) were collected from aquafarm, urban and rural areas in the southern coast of South Korea. Microplastic pollution was widespread in three marine invertebrate species inhabiting the coastal waters of South Korea. Total 1346 microplastics were identified 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 dominant in lugworm. High concentration of microplastics was found in organisms from aquafarm area. Diverse kinds of polymers were identified from urban and aquafarm areas compared 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 microplastics can be overestimated without spectroscopic analysis.

WE132 Spatiotemporal Distribution and Source of Microplastics on Korean Sand Beaches
S. Eo, Korea Institute of Ocean Science and Technology (KIOST); Y. Song, Korea Institute of Ocean Science and Technology KIOST; S. Hong, Korea Institute of Ocean Science and Technology / Oil and POPs research group; J. Lee, J. Lee, Korea Ministry of Oceans & Fisheries Letter Institute of Ocean Science and Technology; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POPs research group

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 researched. 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 programs by virtue of their broad geographical distribution, easy assessiblility, high tolerance to salinity, and limited mobility. Similarly, bivalves can be useful sentinels for the assessment of microplastic pollution in water column because they filter large volumes of seawater, while lugworms can reflect microplastic pollution in sediment bottom. In order to assess the contamination status of microplastics in marine invertebrates and their contamination characteristics according to different feeding habitats and regional sources, mussels (M. edulis), oysters (C. gigas), and lugworms (Perinereis albithuiltes) were collected from aquafarm, urban and rural areas in the southern coast of South Korea. Microplastic pollution was widespread in three marine invertebrate species inhabiting the coastal waters of South Korea. Total 1346 microplastics were identified 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 dominant in lugworm. High concentration of microplastics was found in organisms from aquafarm area. Diverse kinds of polymers were identified from urban and aquafarm areas compared 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 microplastics can be overestimated without spectroscopic analysis.
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/m² 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 determination 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
S. Wagner, Helmholtz Centre for Environmental Research GmbH - UFZ / Department of Environmental Geoscience; C. Schmidt, Helmholtz Centre for Environmental Research UFZ / Department of Hydrogeology; P. Klöckner, Helmholtz Centre for Environmental Research UFZ / Department of Analytical Chemistry

For quantification of plastic with particle sizes filtration, the MPs are analysed by ATR-FTIR in order to study changes in the surface chemistry resulting from photochemical transformation. In both plastics, in the presented study, we sampled suspended and floating matter (with a focus on plastic) along the Elbe River (between the 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
F.R. Storck, DVGW-Technologiezentrum Wasser / Analysis and Water Quality; C. Wiezig, M. Pittroff, DVGW-Technologiezentrum Wasser

Monitoring microplastic particles in surface water requires sampling of large water volumes, typically several hundred litres to several cubic meters. Particle sampling goes hand in hand with particle concentration when a filter system is used. Particle concentration methods in environmental waters consist mainly of natural particles. The high number of natural particles compared to microplastic can disturb the analysis. Besides problems with organic particles, microplastic can be covered by or buried 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 < 500 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 developed. 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. Further is the raise concentration and the characterization of the particles were calculated. 13 Storck, F.R., Kools, S.A.E., Rinck-Plütscher, S. (2015) Microplastics in Fresh Water Sources. 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%20September%20%202015%29.pdf 14 Storck, F., Kitt 2015, Microplastics: 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 Küster, Dr. Oliver Küster, 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
M. Fischer, B. Scholz-Böttcher, Carl von Ossietzky University Oldenburg

There is a raising concern about plastic debris in the environment - 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.

The proportion of microplastics (MP) is expected to increase steadily due to ongoing fragmentation processes. Sources, distribution and accumulation behavior of MP (< 1 mm) 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 highly time-consuming and site-specific.

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
M. Koxi, Wageningen University & Research / Aquatic Ecology and Water Quality Management Group; E. Besseling, Wageningen University & Research Centre / Aquatic Ecology and Water Quality Management Group; C. Kroeze, Wageningen University and Research; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management Group

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 in the aquatic environment. We review the existing 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 debris, we discuss plastic-specific properties, processes and existing mass-balance-, multi-media- and beyond-the-box 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?
A. Pomeroi, International Research Institute of Stavanger / Environment; M. Mæland, International Research Institute of Stavanger (IRIS); A. Vatland Krøvel, International Research Institute of Stavanger; G. Skagerbo, IVAR iks Scandinavia, in the early 1950’s, the development of synthetic 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 threat to the environment. Microplastics can be of either primary or secondary origin. Primary microplastics are purposefully manufactured to be of microscopic size such as plastic micro beads added to face...
high number of natural particles compared to microplastic [1] can disturb the
environment is an essential prerequisite for the assessment of potential ecological
effects. A reliable quantification of the occurrence of plastic material in the aquatic
environment is therefore of great importance, and provides implications and guidance for how fate and transport
behaviour and processes of plastics can be modeled and understood. The models
and their parameters can then be used to estimate the potential ecological impacts of plastic debris, which
are expected to increase in the near future.

The presented study aims to quantitatively assess the occurrence of plastic material in the aquatic
environment and in soil?

The main polymer matrix.

1 mm, plastic particles were separated visually. All extracted particles were
determined to be microplastics. In the presented study, we sampled suspended and floating matter (with a
sampling procedure. Collected samples were fractionated
and analysis. Besides problems with organic particles, microplastic can be covered by
inorganic particles. Based on theoretical calculations and experiments with plastic
particles in the aquatic environment and in soil?

Contamination with plastic debris has been recognized as one of today's major
environmental issues, and its impact on human health and the marine and terrestrial ecosystems is gaining
increasing attention. The ingestion of 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 tried to expand the current knowledge on microplastics in freshwater systems by documenting the occurrence in the digestive system of fish from 15 different species found at 17 different locations in Belgium. Fish were frozen in liquid nitrogen immediately after capture, and later frozen storage until analysis. Fish were tested for microplastic contamination using a combination of methods, including visual inspection and image analysis. The results showed that microplastic contamination varies greatly between species and locations, with some species showing high levels of contamination. Further research is needed to understand the factors influencing microplastic contamination in freshwater ecosystems.

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These findings help to guide experiments with MP under controlled conditions and adds
guidecoals from Flemish rivers are contaminated with microplastics.

G. Mæland, International Research Institute of Stavanger (IRIS); A. Vatland Krøvel,
University of Stavanger, Norway; M. Fischer, B. Scholz-Böttcher, Carl von Ossietzky University Oldenburg
Nordrhein-Westfalen, Germany; S. Wagner, Helmholtz Centre for Environmental Research GmbH - UFZ /
Leipzig, Germany; C. Schmidt, Helmholtz Centre for Environmental Research (02WRS1378F).

WE142 Visual sightings of floating debris in the Mediterranean Sea

C. Cózar, International Research Institute of Stavanger; A. van Wezel, KWR Watercycle Research Institute /
Breda, Netherlands; L. Brouwers, University of Bergen, Norway; M. Mæland, International Research Institute of
Stavanger (IRIS); A. Vatland Krøvel, University of Stavanger, Norway; M. Fischer, B. Scholz-Böttcher, Carl von
Ossietzky University Oldenburg, Germany; S. Wagner, Helmholtz Centre for Environmental Research GmbH - UFZ /
Leipzig, Germany; C. Schmidt, Helmholtz Centre for Environmental Research (02WRS1378F).

WE141 Microplastic contamination in goby (Gobio gobio) from Flemish rivers

B. Slootman, Ceska Vysoká řada, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp / Department of Biology, L. Bervoets, University of Antwerp / Biology; R. Blust, University of Antwerp; C. Belpaire, Research Institute for Nature and Forest INBO

Plastic pollution is continuously growing on a global scale and emerging as a major environmental problem. The ingestion of microplastics by aquatic organisms is an increasing scientific interest towards the habits and effects of this persistent danger. While the smaller sized microplastics (< 5 mm) shown to be omnipresent throughout the aquatic environment, the extent of the problem remains unclear. 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
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These findings help 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 how 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 concentrations of hydrocarbon and aerobic and anaerobic biodegradation of hydrocarbons is well documented, little is known 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. In 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 in concordance 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. Applying 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.

WE145 Exploring inter-species sensitivity to a model hydrocarbon, 2-Methylnaphthalene, 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; the incidence of which has been reported in the coastal area of the North Sea, and the Mediterranean Sea. Furthermore, the biodegradation of hydrocarbons is well documented, little is known 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. In 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 in concordance 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. Applying 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.

WE146 ERA Acute - A multi-compartment environmental oil spill risk assessment model

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 for comparison analyses with other 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: water surface, shallow water, column and seabed. 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 probabilities of exposure, probabilities of lethality and toxicodynamics. 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, including assessment of decommissioning. Sediment structures 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 environmental 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 probability of exposure, probability of lethal effect 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, Ploch 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 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 dispersed (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 the mussel are dependent on exchange processes 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. Novel biomarker responsive enhanced WAF (CE-WAF) of crude oil 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*


In the Baltic area, oil spills are mainly combated 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 traffic threat. 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 PAAH (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 toxic and/or 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

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Steadi 300 mg/kg bw for 28 days. 37% contained genes were newly expressed 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.

**WE151**

Application of time variable toxicity model to single hydrocarbons and petroleum substances

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Many aquatic exposures to petroleum chemicals 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 varying toxicity data 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 embroyos exposed to mixtures of naphtenic 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-stable toxicity data and can help fill this knowledge gap. Application of this framework to existing datasets developed under standard conditions (e.g., constant
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 technologies 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-accommodated 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 mimicking 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 species will be confirmed through molecular analysis as their behavior might be population shifts and hybridization. WAF, both from oil (Troll B) and from oil-dispersant cocktails will be prepared at 10°C using consensus procedures and conditions. However, exposure conditions will vary between 5 and 15°C 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 dimethylphenalene

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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. Toxicoxicokinetic-toxicoodynamic (TMDK) 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 TMDK models are seen as special cases of the GUTS model provided by the General Unified Threshold model for Survival. Here, we apply GUTS to the effects of a single oil component, 1,3-dimethylphenalene, 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 TMDK 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 (ecotox)icity and exposure studies have been conducted after the 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 report the sensitivity of 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 C. elegans 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, namely, Tigriopus japonicus CYP3024A2 and Tj-CYP3024A2 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, these studies suggest that 2007 Iranian IHC oil spill 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 oil.

Keywords: Caenorhabditis elegans, RNAi screening, Zebrafish, Crude oil, Nucleotide excision repair, Mixture toxicity

WE156 Adverse effects of WAFs of crude oil on the Antarctic copepod Tigrigopus kingejoogensis and the temperate congeneric copepod Tigrigopus 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 (Tigrigopus kingejoogensis) and temperate (Tigrigopus japonicus) copepods. Reproductive rates of T. kingejoogensis and T. japonicus were significantly reduced (P < 0.05) in response to WAFs. Furthermore, T. kingejoogensis 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-CYP3024A43 and Tj-CYP3024A42 in response to WAFs, suggesting that CYP genes may contribute to the detoxification mechanism in response to WAF exposure. These findings also suggest that WAFs induce oxidative stress, leading to reproductive impairment in copepods. Furthermore, Tk-CYP3024A43 and Tj-CYP3024A42 genes can be considered as potential biomarkers of WAF toxicity in the congeneric copepods T. kingejoogensis 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 lubricating oils and their highly soluble components, 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/estuarine benthic meiofauna were exposed to dispersed oil for 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 recovery in the highest concentration treatments of freshwater microcosms. 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/oil nematodes where compared.

**WE158 Screening methods for assessing toxicity and fate of produced waters**

E. Michie, SHELL / Shell Health; C.V. Eadsforth, Shell International / Shell Health; G. Whale, Shell Health / Shell Health Risk Science Team; M.G. Smit, Shell Global Solutions Inc.

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 screening methods provide a quick and efficient method of obtaining effective and 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® 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 and amongst other factors, and bioaccumulation potential is of low concern (BCC < 0.001) 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**

M.J. Bock, H. Robinson, Ramboll Environ; R. Wenning, Ramboll Environ / Ecology & Sediment Management; D.P. French-McCay, RPS ASA; J.J. Rowe, RPS/Applied Science Associates; A. Hayward Walker, SEA Consulting Group during the Deepwater Horizon accident, subsea dispersant injection (SSDI) was used as a countermeasure for this uncontrolled release of a deep sea 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 judgments 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 trade-offs 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 surface 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. SIMA quantifies the response technologies impacted 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 project team, comprising 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**

A. Faber, Copernicus Institute - Utrecht University / Environmental Sciences; M.P. Annevelink, KWR Watercycle Research Institute; P.P. Schot, Copernicus Institute Utrecht University / Environmental Sciences; K.A. Baken, KWR Watercycle Research Institute / CWG; M. Schrits, E. Enke, KWR Watercycle Research Institute; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Science; P. de Voogt, University of Amsterdam / IBED; A. van Welzel, KWR Watercycle Research Institute / Chemical Water Quality and Health Publicly available chemical risk assessments of hydraulic fracturing are generally based on shale gas related studies. The industry in the U.S. has already started to develop 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 gas chromatography/MS, the latter of which allowed for the differentiation of the main 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 mg/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 the estrogen and anti-estrogenicity for fracturing fluids and flowback. The results point to the importance of the development of reliable test systems that can help to detect substances that are bioavailable and measure their exposure 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**

T. Seiler, RWTH Aachen University / Ecosystem Analysis; S. Johann, Institute for Environmental Research Biology V; L. Nuesser, RWTH Aachen University, Institute for Environmental Research / Department of Ecosystem Analysis; K.K. Lehto, A. Aho, R. Turia, A. Rumvall, CALUX Finnish Environment Institute / Marine Research Centre; I. Marigomez Allende, University of the Basque country UPV/EHU / Dept of Zoology and Animal Cell Biology; M. Soto, University of the Basque Country / Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology PIEUP/EHU; N. Etxebarria, University of the Basque Country / Zoology and Animal Cell Biology, Research Centre for Experimental Marine Biology and Biotechnology PIEUP/EHU; S. Pärt, Tallinn University of Technology; S. Pärt, Tallinn University of Technology / Marine System Institute; E. Emke, KWR Watercycle Research Institute / CWG; M. Schriks, E. Emke, KWR Watercycle Research Institute / CWG; M. Schriks, E. Emke, KWR Watercycle Research Institute / CWG; M. Schriks, E. Emke, KWR Watercycle Research Institute / CWG; M. Schriks, E. Emke, KWR Watercycle Research Institute / CWG.

Publicly available chemical risk assessments of hydraulic fracturing are generally based on shale gas related studies. The industry in the U.S. has already started to develop 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 gas chromatography/MS, the latter of which allowed for the differentiation of the main 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 mg/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 the estrogen and anti-estrogenicity for fracturing fluids and flowback. The results point to the importance of the development of reliable test systems that can help to detect substances that are bioavailable and measure their exposure to the handling, transport and treatment of hydraulic fracturing related waters to avoid adverse environmental and human health impacts.
Marine Research Centre, H. Hollert, RWTH Aachen University / Department of Ecosystem Analysis

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 technologies, 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 comparing the evaluated changes to previously reported information. Regional specimen will be sampled from representative locations in the Baltic Sea and northern Atlantic. Bivalves will further 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 will be tested in the bioavailability biotesting and oil spill response technologies 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 PETROTOX model and a data gap analysis, threshold values will be derived. The overall objectives are the improvement of the composition of the surrounding soil environment, for each borehole 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 depths. 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)

WE163 Migration of polycyclic aromatic hydrocarbons in vertical profile of alluvial sediments of the Sava River, Serbia
T. Solevic Knudsen, ICHTM; M. Blic, ICHTM / Department of Chemistry; S. Bulatovic, Faculty of Chemistry, University of Belgrade; G. Devic, J. Milic, Institute of Chemistry, Technology and Metallurgy, University of Belgrade; S. Miletic, Institute of Chemistry, Technology and Metallurgy, University of Belgrade / Department of Chemistry; M.M. Vrivic, Faculty of Chemistry, University of Belgrade / Faculty of Chemistry

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 oil and sediment samples were taken from several depths: 0-0.30 m; 0.50 m; 1.00 m; 1.50 m; 2.00 m; 5.00 m; 7.00 m, 10.00 m, 12.5 m and 15.00 m. Pedologic analysis revealed that lithologic profiles are created 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 oil 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; Jovanovic 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 total amount 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 mobilized 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
M. Blic, ICHTM / Department of Chemistry; S. Bulatovic, Faculty of Chemistry, University of Belgrade; T. Solevic Knudsen, ICHTM; J. Avdalovic, Institute of Chemistry, Technology and Metallurgy, University of Belgrade / Department of Chemistry; J. Stefanovic – Kojic, Institute of Chemistry, Technology and Metallurgy, University of Belgrade; S. Miletic, Institute of Chemistry, Technology and Metallurgy, University of Belgrade / Department of Chemistry; M.M. Vrivic, Faculty of Chemistry, University of Belgrade / Faculty of Chemistry

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 pollutants in sediments and soils of the site located in the New Belgrade oil heating plant. The soil was sampled from 20 micro locations and at 5 different depths (down to the depth of 2 m) making in total 100 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; Jovanovic 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 depths. 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
H. Barmentlo, M. Schrana, CML Leiden University / Conservation Biology; E. Hunting, CML Leiden University; M.G. Vijver, CML Leiden University / Conservation Biology

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 mosescan data
A. Rigó, IMDEA Water Institute / Aquatic Ecotoxicology; P. van den Brink, Alterra and Wageningen University

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. As these data can be highly variable (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 unimodal 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 species that are associated with 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
A. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change; P. Thörbek, Syngenta / Environment and Safety; M.C. Finnegan, Syngenta; T. Strauss, Research Institute gaia - Research Institute for Ecosystem Analysis and Assessment; H. Barmentlo, gaiac - Research Institute for Ecosystem Analysis and Assessment; M. Schrama, CML Leiden University / Conservation Biology; E. Zimmer, IBACON GmbH; S. Augustine, Akvaplan-niva; A. Gergs, gaiac - Research Institute for Ecosystem Analysis and Assessment / Department of Environmental, Social and Spatial Change; T. Preuss, Bayer Ag / Environmental Safety; V. Ducrot, Bayer CropScience AG / Environmental Safety. Ecotoxicology 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 unstressed species and unstressed ecological scenarios or exposure situations, which will improve the quality of ERA as well as saving time and resources. Several traits, 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 upcoming cycles of the regulatory process. The TKTD 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 develop a sublethal metabolism and interactions model 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 so-called BEEHAVE model was 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 these 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 compare the realistic landscape with spatially resolved weather data, and data on seasonal nectar and pollen availability, which represents the highest required level of detail. 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
T. Schad, Bayer Ag / Environmental Modelling; Z. Gao, Bayer AG Crop Science Division; S. Bub, Tier 3 Solutions; A. Chapple, Bayer Crop Science AG; T. Preuss, Bayer AG Crop Science Division. 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-deposited depositions on 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 we have 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 model 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
A. Maseddu, A. Barausse, L. Grechi, A. Pavita, L. Palmeri, University of Padua / Department of Industrial Engineering; D. Baldan, University of Padova / Department of Industrial Engineering. Aquatic ecosystem models are useful tools that can improve the ecological realism of chemical risk assessment with respect to the conventional approaches, which are essentially descriptive ecological models, threshold and no-effect concentration (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. Polychlorinated Biphenyls (PCBs) 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 Po basin. The comparative assessment of the impact of untreated wastewater on a major European river evaluated with a virtual effect-directed analysis (vEDA) approach Rapid identification of neuroendocrine-disrupting chemicals in fish using a biomarker - virtual effect-directed analysis (vEDA) approach

Assessing the risk of endocrine-disrupting chemicals (EEDCs) is important, as such compounds can cause a range of health effects. A recent study evaluated the 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 Po basin. The comparative assessment of the impact of untreated wastewater on a major European river evaluated with a virtual effect-directed analysis (vEDA) approach.

**Integrated approaches for linking chemical contamination with biological effects**

**WE172**

In situ effects of untreated sewage discharged into the River Danube: expression of selected genes in liver of caged common carp D. Tenj, S. Sipos, University of Novi Sad Faculty of Sciences / Biology and Ecology; B. Micić, Faculty of Biology / Department of Biology and Ecology, Laboratory of Ecotoxicology (LETOX); V. Knezevic, Faculty of Sciences / Department of Ecology and Biology; S. Kasiarević, University of Novi Sad, Faculty of Sciences / Department of Biology and Ecology, Laboratory of Ecotoxicology (LETOX); L. Teodorovic, University of Novi Sad / Department of Biology and Ecology, Laboratory of Ecotoxicology (LETOX); W. Brack, Helmholtz Centre for Environmental Research UFZ / Effect Directed Analysis

The impact of untreated wastewater on a major European river was evaluated with a virtual effect-directed analysis (vEDA) approach to investigate the accumulation of environmental 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 carp (Cyprinus carpio) collected during a caging experiment carried out at the discharge point of the respective contamination source. The chosen site had previously been 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-disrupting 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 nervous system were selected 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…) in rainfall events drained these contaminated sites, 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 biogeochemical processes, organic matter recycle…). The bioturbation, which is defined as the mixing of sediment with overlying water, is carried out 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 organ), and (iii) the antioxidant defense systems upstream and arable land use could be observed. While pharmaceuticals and other typical contaminants were underrepresented with grab samples. Nevertheless, occasional concentration peaks were observed. These compounds were still lower than their corresponding EC50 levels reported in literature. Therefore the observed immobility could not be explained by the presence 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.

**WE175**

Screening of Dutch surface water quality using Daphnia magna bioassays

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Risk assessment and remediation of mine sites and contaminated sediments and plant material in constructed wetlands receiving hyperalkaline (pH > 9.5) steel slag leachates.

**WE173**

Rapid identification of neuroendocrine-disrupting chemicals in fish using a biomarker - virtual effect-directed analysis (vEDA) approach

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Virtual effect-directed 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 uncover contaminants co-varying with mutagenicity in water samples. Virtual EDA is applied here to investigate the accumulation of environmental 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 carp (Cyprinus carpio) collected during a caging experiment carried out at the discharge point of the respective contamination source. The chosen site had previously been 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-disrupting 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 nervous system were selected 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.
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 developing new bioassays. Sixteen 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 to determine the EC50 and a linear model was applied to compare immobilization means of the samples with the corresponding controls. If dose response relationships were obtained in dilution series, EC50 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 one sample from an Amsterdam harbour and from a green house area caused significant daphnid immobilization after 48h. From the green house area water sample dilution series the EC50 value was calculated to be 97.5%. Previous chemical analysis by the responsible waterboard showed that the green house site contained relatively high concentrations of naphthalene, fluorene, anthracene, 44-DDT and 44-DDE. Yet, the concentrations of the measured compounds were still lower than their corresponding EC50 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 daphnids test was performed at all locations. Subsequent to field exposure, accumulated compounds were extracted from the PS after which a battery of 3 in-situ, 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 chemicals that are used, among others, to reduce the prevalence of pathogens. 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 bioassays being done by SPEAR index and 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 sampling events 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 high 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. However, no information on the 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**

W. Hayes, G. Humes, University of Hull / School of Environmental Sciences.

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 (< 13) steel slag leachate. Steel slag leachates are dominated by NaOH 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 was little 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**

O.L. W. wc consulting: A. Peters, wc; G. Merriman, WCA Environment
Limited; A. Boullent, Rio Tinto / Legacy 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
sites have been declared clean. While in situ monitoring is recommended if it can be adequately
utilized 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
reviews recommendations to the water monitoring programme that can be made from
our analysis of water quality data. The use of agrochemicals is crucial to modern agriculture. In fact, fertilization
of soil, while in situ in the presence of vegetation. The use of specific remediation measures is strongly
amended favored decreasing Eh values, increasing metal mobility, especially
than MWR providing nutrients. Under hydric conditions (salt marsh) B and L
increased water holding capacity and reduced metal mobility, but was less effective
in the germination of the present seeds. Plants accumulated more metalloid(s) in
sites which do not pose any potential risks to the local environment are identified,
and recommendations are made for future monitoring activities and sites at which cannot currently be identified as posing no risk.

WE181
Ecological impacts and ecosystem response in an old mining area of SE Spain:
Informing lessons for phytoamangement
J. Alvarez-Regueiro, H. Conesa Alcarzar, I. Párraga-Aguado, O. Martínez Oró, M.
Tercero Gómez, A. Peñalver Alcalá, Escuela Técnica Superior de Ingeniería
Agronómica, Universidad Politécnica de Cartagena / Ciencia y Tecnología Agraria;
F. Jiménez-Cárceles, TECOMA, INGENIERÍA Y AMBIENTE S.L.; J. Martínez-Sánchez, Escuela Técnica Superior de Ingeniería de la Universidad Politécnica de Cartagena / Producción Vegetal; M. González-Alcarzar, Department of Biology & CESAM - University of Aveiro / Biology & CESAM
This presentation shows the results of a study in a field which 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 (tailing and polluted salt marshes) to take advantage of the natural
processes for phytomanagement purposes. Field works studied the environmental
gradients, soil-plant relationships, plant-favourable edaphic niches, key species,
seed bank and metalloid accumulation in soils and plants. Greenhouse works gave
information 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
marsh the most saline and wettest sites were the most affected by the mine wastes,
pioner vegetation (grasses and shrubs), improving the nutritional and
former mining site
in the presence of vegetation. The use of specific remediation measures is strongly
amended favored decreasing Eh values, increasing metal mobility, especially
than MWR providing nutrients. Under hydric conditions (salt marsh) B and L
increased water holding capacity and reduced metal mobility, but was less effective
in the germination of the present seeds. Plants accumulated more metalloid(s) in
sites which do not pose any potential risks to the local environment are identified,
and recommendations are made for future monitoring activities and sites at which cannot currently be identified as posing no risk.

WE183
DETERMINATION OF POLLUTION LEVELS OF SOME HEAVY METALS IN THE COAL MINING ENVIRONMENT OF UDI, AKWUKE AND NGWO COMMUNITIES OF ENUGU STATE, NIGERIA
C.K. Uhama, Anambra State University / Biochemistry
SOIL, WATER AND FOOD CULTIVARS IN THE COAL MINING COMMUNITIES IN ENUGU STATE WERE INVESTIGATED FOR THE
WITH THESE HEAVY METALS. THE LEAFY VEGETABLE (PUMPKIN AND
snail Cantareus aspersus. Calculation of the SET indice is simple: SETindice =
(Σ-AQ)-1, with AQ (accumulation quotient for each metal = [Concentration in snails caged for 28 days on the site]/ [Internal concentration of reference: C[Ref]
The aim was to rank the management priorities of the 8 plots of the site based on
laboratory analyses of the total TMs and the bioavailability of the TMs (indicated by
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, Ti 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 of 8.6 mg kg⁻¹). SETindice and the SETindice was 924 based on the analysis of 14 TMs. Previous studies using the SET
indice only reached SETindice of 7.7, showing the very high bioavailability of TM on the studied site. Comparison of the SETindice allows ranking the plots according to the biological assessment of bioavailability of TMs; the biological ranking does not match the Heaking based ranking of bioavailability 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.

WE184
Long-term coal mining activities in Enugu metropolis; could it be responsible for
bioavailability of heavy metals in plants?
A. Onwurah, UNIVERSITY OF NIGERIA, NSUKKA / Environmental Management; K. Uhamo, M. Ifemeje, Chukwuemeka Odumegwu Ojukwu University, Uli, Anambra State, Nigeria. / Department of Biochemistry; I.C. Jonathan, Anambra State University / Biochemistry
Coal mining activities which spanned over a period of 48 years 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 distribution, we examined the bioavailability 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 metal levels. Parallel
control experiments were run using similar soil samples devoid of coal particulates,
but treated with different levels of the heavy metal. The samples used in this study are yam (Discorea rotundata), cassava (Manihot esculenta), scented leaf

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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
D.A. Sarigiannis, Aristotle University of Thessaloniki / Chemical Engineering; I. Furdhi, University of Thessaly / Department of Biochemistry and Biotechnology; S. Karakitsos, Aristotle University of Thessaloniki / Chemical Engineering; A. Tsatsakis, University of Crete; K. Golokhvat, Far Eastern Federal University
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 kidney cells, testes and other organs. Pathways of specific responses in each tissue. It has also been 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±1.2). Venuous 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 µg/m³, 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 inflammatory and fibrotic processes showed that impurities influence significantly the induction of key toxicological pathways such as inflammation mediated by chemoactive 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 and fibrotic responses. Changes observed in 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 evaluate 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 increase crop efficiency and lead to a better quality of product recovery in agricultural activities. However, the number of compounds has increased exponentially throughout the world in the recent years and it has caused environmental problems, such as water, soil and air pollution. Because of these reasons, the reduction in chemicals used and their replacewith environmentally-friendly compounds has emerged as one 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 fertilizer and increase their efficiency on crops. Nevertheless, one of the most common compounds used in those products is nanoZnO which 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 withnanoZnO compound. The study will start with the definition of the ecotoxicological parameters (NOEC, EC50) for NMs released from fertilizers soil matrices and soil leaches, upon non-target organisms (eg. microbial communities, earthworms etc.), which are necessary to understand the impact of nanofertilizers. Moreover, the risk upon human health due to the emission from these agrochemicals, and their degradation and interaction 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 products throughout their life cycle, will be applied to assess the environmental performance of nanofertilizers with 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 both ecotoxicological and human risks.

WE187 NanoCRED: A transparent framework to assess the regulatory adequacy of ecotoxicity data for nanomaterials - relevance and reliability revisited
N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment; H. Lützhoff, LEO Pharma / DTU Environment; M. Agerström, Stockholm University / Environmental Science and Analytical Chemistry; A. Baun, DTU Environment / DTU Environment
Environmental hazard and risk assessment is the foundation for regulatory decisions to protect the environment from unintended adverse effects caused by chemicals and 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 following Good Laboratory Practice (GLP). It is known, however, that engineered nanomaterials behave very differently in ecotoxicity tests 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 lack of proper test guidelines for testing NM is a major obstacle to the use of NM, whereas 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 guidelines and read across between NMs, we are assessing, and 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 experimental ecotoxicological studies and not only immunological tests but also morphological 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 (alga, daphnia and zebrafish embryo) and terrestrial organisms (earthworm, brownflycatcher).
WE189
Grouping of nanomaterials regarding aquatic ecotoxicity - hypotheses for selection of 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, CeO₂ and TiO₂ were included. Several forms per NM-type were considered differing mainly in size, shape, crystalline structure, surface area, solubility and zeta-potential. Specific surface characteristics were included. As such, alkylamine modified 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 text media. It was obvious that mono-causal relationships do not exist. For ion-releasing NMs the solubility is discussed as relevant parameter regarding ecotoxicity. 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 TiO₂ and CeO₂. 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 CeO₂ 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 spp.
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The fusing of biomolecules and organisms onto aquatic surfaces is responsible for undesiable species dispersion, surface corrosion, and increases fuel consumption of engines. Use of Deicide DCOIT (4,5-Dichloro-2-occtyl-2H-isothiazol-3-one) is considered an environmentally acceptable method of preventing marine species attachment (European Directive 98/9/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 reef-building corals and other marine invertebrates. Elevated seawater temperatures, as predicted under global climate change scenarios, are known to have strong negative effects on Symbiodinium spp. DCOIT is also a known antagonist of photosynthesis, and thus there is the potential for a joint effect of the two stressors. Toxicity assays were performed by exposing Symbiodinium strains Ag3p1 and 2464 to a range of 5 concentrations (15 - 240 µg L⁻¹) of empty silica nanocontainers (SiNC); SiNC©/DCOIT and DCOIT at two different temperatures (26 and 30.5°C) for 72h. Photophysiological parameters (Fv/Fm, ETRcalc; 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 DCHF-DA respectively, while growth inhibition was access according to the OECD 201 guideline. After 24h, maximum quantum efficiency of photosystem II (Fv/Fm) reduction of 20% was observed at 30 µg L⁻¹ or 60 µg L⁻¹ DCOIT in 2464 and Ag3p1 strains respectively, but recovery could not be observed up to 96h. Further, 50% growth inhibition rate (IRr) was evident at 12.5 µg L⁻¹ (DCOIT) compared to SiNC©/DCOIT (50% at 23 µg L⁻¹), showed strong DCOIT toxicity. The SiNC present had no effects at lower concentrations, but shows 50% Ir above 60 µg L⁻¹. The growth effects can also be assessed in terms of cell cycle progression and ROS levels, improvements in capacity to address toxicological responses at population level.

Keywords: Nanotechnology; Photophysiology; Global changes; ROS

WE191
Nano SARA: 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 environment and human health. The Nano SARA 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, determined by different parameters of interest. - Toxicological tests especially in sediment-dwelling organisms (bioaccumulation, growth inhibition, development delay and teratogenicity) and Heterocycris incongruens (growth inhibition). The nanoparticles were selected on the basis of different nanoparticle 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
S. Lekamge, A. Miranda, R. Shukla, RMIT University / School of Science; D. Nugegoda, RMIT University / School of Applied Sciences

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 may alter the biological uptake, and lethal and sub-lethal effects on organisms. We followed an interdisciplinary and multidisciplin ary approach to investigate the effects of silver NPs coated with three different ligands; Tyrosine (T-AgNP), Epligogallategallate (E-AgNP) and Curcumin (C-AgNP) in relation to the toxicity to a key aquatic organism; Daphnia magna. 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 unicellar 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 LC50 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
R. Weltens, F. Beutels, VITO; L. Geerts, VITO NV; S. Verstraelen, VITO NV / Health
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: 1µg of each ink was suspended in 11 demineralized 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 tested inks of the varnish (ink without 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 (EC50 values were respectively 0.037% and 0.033% leachate for the 2 prototypes) and algae (EC50 values were 0.15% and 0.23% leachate for the 2 prototypes). These EC50 values are equivalent to 1.35 and 0.861 µg/l Ag (Daphnia) 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 higher ecotoxic levels. This has to be taken into account in further product development e.g. 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
I. De Leersnyder, L. De Gelder, Ghent University / Department of Applied Biosciences; I. Van Driessche, Ghent University / Department of Inorganic and Physical Chemistry; P. Vermeir, Ghent University / Department of Applied Biosciences
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
S. Polydorou, Incheon University; J. Park, Univ of Incheon / Biology; Y. Kim, Incheon National University / Department of Marine Science; T. Han, Incheon National University / Marine Science
The rapid response of the aquatic macrophyte, Spirodela polyrhiza, to toxicants was studied by examining chlorophyll 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 0.0045 0.09 0.18, 0.36 and 0.72 mg L-1 of antimony, values of Y(NPQ) showed a decrease, which was 0.509, 0.505, 0.448, 0.478, 0.319 and 0.373 respectively. In response to varying concentrations of 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 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
F. Pacchioni, ENEA; s. chiaro, ENEA CR; S. Scalbi, ENEA / UTVALAMB- Technology Unit Model, Small Materials and Technologies for the Environmental A; S. manzo, ENEA; P. Buttoli, ENEA / Territorial and Production Systems Sustainability Department; G. Barberio, ENEA
Nanotechnology represents an important technological option, useful to improve sustainability. It is critical, however, to properly define the needed conditions so that these nanomaterials (NMs) do not affect the environment and the public. This is especially true for aquatic organisms. The development of eco(n)toxicological models is necessary to bridge the gap between theoretical and experimental data. In this paper, we discuss the need for a consistent and coordinated approach that guarantees the best possible approximation to the current knowledge on the nanomaterials and the entire life cycle. To this aim it is essential to perform Environmental Risk Assessment (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 internal 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
E. Semenzin, Ca Foscari University of Venice / Department of Environmental Sciences Informatics and Statistics; s. subramanian, Ca Foscari University of Venice / Dept of Environmental Sciences Informatics and Statistics; A. Zabeo, University Ca Foscari Venice; L. Pizzol, University Ca Foscari of Venice / Basei, University Ca Foscari Venice; W. Fransman, TNO; T. Wilkins, University of Leeds; D. Hristozov, Ca Foscari University of Venice; A. Marcomini, University of Venice / Department of Environmental Sciences Informatics and Statistics. The exposure to global nanotechnologies is expected to reach 4.4 billion 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 intended to develop 'green' products and applications. The activities of ECHA’s working groups 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 development of appropriate Technological Alternatives and Risk Management Measures (TARM) 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 prescription (e.g. European Commission’s Policy and Strategy for Nanotechnology). 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 TARM) 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 conventional risk assessments are available.

WE198 Human Health Risk Assessment along the life-cycle of nano-enabled products

L. Pizzol, UNIVE / Environmental Sciences, Informatics and Statistics; D. Hristozov, Ca Foscari University of Venice; A. Zabeo, University Ca Foscari Venice; E. Semenzin, Ca Foscari University of Venice / Environment of Environmental Sciences Informatics and Statistics; v. subramanian, Ca Foscari University of Venice / Dept of Environmental Sciences Informatics and Statistics; G. Basei, University Ca Foscari Venice; W. Fransman, TNO; J. Gosens, RIVM / DMG; F. Cassée, RIVM; J. Kovisto, K.A. Jensen, National Research Centre for the Working Environment; V. Stone, Heriot-Watt University / Hazardous Waste and Toxic Substance Reduction; S. Vanpee, RIVM; S.F. Oostgoed, Denmark DTU / DTU Environment; W. Wohllibben, BASF; A. Marconimi, University of Venice / Department of Environmental Sciences Informatics and Statistics

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 stages (exposure, formation, 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 lifetime stage, 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 LUA). 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 (TiO2) 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

D. Kühnel, Helmholtz-Centre for Environmental Research / Bioanalytical Ecotoxicology; S. Reithel, UFZ Leipzig / Environmental Sciences Informatics and Statistics; v. subramanian, Ca Foscari University of Venice / Dept of Environmental Sciences Informatics and Statistics; G. Basei, University Ca Foscari Venice; W. Fransman, TNO; J. Gosens, RIVM / DMG; F. Cassée, RIVM; J. Kovisto, K.A. Jensen, National Research Centre for the Working Environment; V. Stone, Heriot-Watt University / Hazardous Waste and Toxic Substance Reduction; S. Vanpee, RIVM; S.F. Oostgoed, Denmark DTU / DTU Environment; W. Wohllibben, BASF; A. Marconimi, University of Venice / Department of Environmental Sciences Informatics and Statistics

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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, BODs, microbial load, and toxicity as well as high concentrations of particular matter, proteins, starch, fat, oils and grease, detergents, disinfectants, and pharmaceutical products. Therefore, assessment of the used nanomaterial in any given setting (products, humans, includes mandatory and desirable assessment criteria covering the topics and knowledge on nanomaterials synthetic nanomaterials developed in the last decade together with all current and supporting the environmental risk assessment of nanomaterials with the results of the application of the developed HHRA methodology to the (TiO2) for self-cleaning coating for ceramic tiles. The focus of this contribution stage as well as for the entire lifecycle considering all lifecycle stages, targets, lifecycle stage, a specific target, a specific activity (where applicable) and a defined on data availability. Risk is estimated for a combination of aspects including a (hazard information) and exposure models. The outputs of these models, as well as mechanisms understanding remains limited, and there is also no simple correlation conservative risk estimations are available. Are there significant acute ecotoxicological effects of nanoparticles? WE205 Life cycle thinking for the Brussels’ circular economy transition W. Achten, Université Libre de Bruxelles / Geoscience, Environment and Society; J. Beliën, KU Leuven / Onderzoeksgroep Kwantitatieve Bedrijfsprocessen (KBPRo); P. De Bruucker, KU Leuven / Centre for Economics and Corporate Sustainability (CEDON); M. Degrez, Université Libre de Bruxelles / Materials engineering, characterization, synthesis, and recycling (4MAT); S. De Jaeger, KU Leuven / Centre for Economics and Corporate Sustainability (CEDON); J. Eyckmans, KU Leuven / Faculty of Economics and Business Development; C. Lavigne, KU Leuven / Centre for Economics and Corporate Sustainability (CEDON); E.B. Towa, Université Libre de Bruxelles / Recycling and Environment; V. Zeller, Université Libre de Bruxelles 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 analysis of waste streams in the BCR. A key research question is to determine whether a question about 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 well as for the target situation of the future waste management scenario. Further, the results of these LCA as well as 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 in 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 A. Szilágyi; R. Sándorné Bodnár; G. Gröllér; K. Szita Toth, University of Miskolc / SETAC Europe 27th Annual Meeting Abstract Book 275
WE207 Recent advances in Life Cycle Assessment for soil and sediment remediation
C. Cavaye, KV Leuven / Centre for Economics and Corporate Sustainability
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 severely 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 assessment method used and that the choice of impact categories heavly 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 assessments 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 LCAs increasingly evaluates 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
C. Pereira, T.P. da Costa, P. Quinteiro, L. Arroja, A. Dias, University of Aveiro / Department of Environment and Planning
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 energy production and consumption, which is strongly connected 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 interfere with 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 a product's or process's sustainability for decision-making. 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 kW by converting eucalypt residues delivered to the power plant. System boundaries include the stage of forest management, forest 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 of this study provide an improved impact assessment of a wide range of variation when different management scenarios and wood productivities are taken into account. Finally, the main environmental decisions and current policy negotiations 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
T. Schaubroeck, Luxembourg Institute of Science and Technology (LIST) / Research Group EnVOC, B. Rugani, Luxembourg Institute of Science and Technology (LIST) / Environmental Research & Innovation (ERIN)
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 focused on the impacts of a studied system 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 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. Turned out that recycling is a component into a reusable system 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 operation have proved to be both economically and environmentally sound. In the liminal and environmental point of view. However, it must be considered that in preparing suitably clean cullets for recycling in the glass process, 25 to 35% 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 technologies from a feasibility, environmental and economical point of view. The aim of this study is to assess the environmental performance of expanded
Embedding Sustainable Strategy into Innovation: L’Oréal’s sustainable product assessment tool

P. Martz, L’Oréal Research & Innovation / LOREAL; j. campion, L’Oréal Research & Innovation; J. Lharidon, L’Oréal Research & Innovation / Life Sciences Direction; L. Gilbert, L’Oréal Research & Innovation; P. Bonningue, LOREAL; M. Vargas González, F. Witte, D. Caudrelier, Quantis

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: (1) Product Innovation, (2) Supply Chain, (3) Financial Performance, and (4) 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 provides a powerful tool to understand sustainability metrics into day-to-day innovation: Product designers will be able to assess if a new design complies with L’Oréal sustainability strategy.

Life Cycle Assessment in Practice - Helping SMEs to integrate life cycle approach into everyday work

S. Groslandt, University of Liège - Chemical Engineering / Dpt of Chemical Engineering - PEPs; A. Leonard, University of Liège

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 aim of the Life Cycle in Practice project is to develop methods to help SMEs to adopt Life Cycle Assessment (LCA) tool within their organization and to start thinking about the environmental impacts of their products and services. The project has a pioneering character and a great potential to help SMEs to start thinking about the environmental impacts of their products and services. 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The project has a pioneering character and a great potential to help SMEs to start thinking about the environmental impacts of their products and services. The project has a pioneering character and a great potential to help SMEs to start thinking about the environmental impacts of their products and services. The project has a pioneering character and a great potential to help SMEs to start thinking about the environmental impacts of their products and services.
hosting one of the Physical Resource Centres in the University of Liège to welcome SEMs 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.lcip.eu/. The challenges of Life Cycle Sustainability Assessment (LCSA) of energy technologies (P)

WE215 Sustainability issues of a new building integrated PV system D. Garraut, I. Herrera, C. De la Rúa, N. Caldés, I. Rodríguez-Serrano, Y. Lechon, CIEMAT / Energy Dpt Energy Systems Analysis Unit REELCOOP, an EU-FFP 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 WE216 Exploratory analysis of the potential effects of criticality on solar and wind energy technologies in Spain D. Garrain, N. Caldés, CIEMAT / Energy Dpt Energy Systems Analysis Unit 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 I. Herrera, C. De la Rúa, N. Caldés, I. Rodríguez-Serrano, A. Gamarra, Y. Lechon, CIEMAT / Energy Dpt Energy Systems Analysis Unit A sustainability life cycle assessment of various biofuel 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 K. J. Schieder, ERM; S. Crews, Gerelia; S. Aumonier, ERM 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 siliconmanganese have been omitted. This approach leads to an underestimated environmental impact footprint 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 mild-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 environmental 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. One reason is that 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 Liège / Chemical Engineering; A. Leonard, University of Liège; E. Pirard, University of Liège / Department Argenco Resources, and more specifically mineral and metal resources, are on debate concerning how to model them in a LCA approach. Impact assessment based on life cycle assessment (LCA) 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 involves the study of the ventilation effect in PV façades. This prototype is currently installed at Yazar University (Izmir, Turkey - 2016). This work presents WE222 Comparison of methods for dealing with multi-functionality in a recycling context
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 products or components with multiple functions. Recycling of PV 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 common approaches for dealing with multiplicity in LCA are identified. This review will also focus on the different interpretations that practitioners give to the same named methods. For instance, ‘system expansion’ is assimilated by some practitioners to ‘substitution’ or ‘credit awarding’. However other practitioners make a clear distinction between these two actions: including the multifunctionalities in the functional unit and considering recycling as a separate step. The study will highlight advantages and trade-offs related to chosen methods. Although the cut-off method is straightforward 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 amount 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 assumptions, such as the attribution of 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 recovery and recycling of valuable metals (silver and copper).

WE225 Assessing selected metals flows in France and their recycling potential
A. Thevenot, Université de Bordeaux; P. Loubet, CYI-ISM / ISM CyVi; G. Sommann, University of Bordeaux / ISM CyVi
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.

In a current study, 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 and its values are the changes in resources 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. In a next step, 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 and its values are the changes in resources 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.

Historically, treating low-grade primary ores and secondary industrial residues often yield significant amounts of critical raw materials. 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 SUITOT disaggregation. We provide a first attempt for obtaining data stemming from input-output tables and processes linked together by MFA in order to deal with changing metal flows in France. Based on the data, foresee to evaluate the potential of increasing secondary raw material sources.

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
Y. Wang, J. Wang, J. Mu, X. Ma, National Marine Environmental Monitoring Center China

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) for two 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 of 7.94 μg/L at 234 mg/L, and 3.9 μg/L, and NP with values of 0.22 and 3.9 μg/L for NP-related and non-NP-related endpoints.
We227 Testing and assessment of endocrine disrupting effects of chemicals under Japanese programs: from EXTEND2010 to EXTEND2016
K. Yamazaki, Ministry of 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 specific 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 be accelerated. Effects of chemicals substances, establishment of assessment procedures and implementation of assessment should be accelerated,” relevant activities should be accelerated.

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. Systematic 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 Amino 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
Amino 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 a number of 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 acute 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 steriodogenesis 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 where similar AO surfactant showed no significant effects 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 (VGT). There were no effects on sperm motility, secondary sexual characteristics, tubercle scores or VGT. 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 full test orders under the Tier 1 EDSP. As the initial group was 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 steriodogenesis. These findings are fully consistent with peer-reviewed studies using functionally equivalent in vitro assays and predictions from structure activity relationships. Additionally, the in vivo 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 mammalian reproduction assays, glyphosate exposure had no impact on estrogenic, androgenic, steroidogenic and thyogenic 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 the VGT if an existing fish 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
D.B. Huggett, EAG Laboratories / Department of Biological Sciences; M. McCooe, L.S. Ortigo, Bayer CropScience / Environmental Toxicology and Risk Assessment; K.S. Henry, NovaSource/Tessenderlo Kerley, Inc.
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 in the 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 conducted 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 that 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, the 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?
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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 effects(s) are dependent on the actual internal concentration within the organism, their homeostasis can easily be disturbed by external influences, thereby eliciting detrimental effects related to respective organ or species 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 present in the organism and the used experimental 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 Disruptor 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.

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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), Polycyclic aromatic hydrocarbons (PAHs), 2,4-D, 2,4-Dichlorophenoxyacetic acid and its derivatives (PFOs), dioxins and dioxin-like compounds, heptachlor, heptachlor epoxide, hexabromocyclododecane (HCDDC), 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). Data obtained for each species in each studied site 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

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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 previously impaired due to elevated contaminants. We present an evaluation of 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 compensate for environmental contamination. The results of a systematic review and meta-analysis of the literature, 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

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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 a weight-of-evidence approach. This new paradigm of risk assessment, risk management, and decision making for sediments of any nature allows the environment-regulator to consider a range of chemical mixtures within the sediment toxicant weight-of-assessment approach for Australia (Simpson and Batley, 2016) has been evaluated for application in environmental regulation. We have evaluated the potential of the new approach, which includes more static toxicity, ecology, bioaccumulation and other approaches including biomarkers, and recommended that it be adopted within the environmental regulatory framework in Victoria for freshwater, estuarine 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 applied. Characterisation of sediment toxicity. 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 mortality for sediment samples collected in the Swarte Schelde, 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 systematic review and meta-analysis of the literature, which includes more static chemical water systems, and for brackish sediments in the different ecotopes of the Scheldt estuary.
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. Besides 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 (Irsee) 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 (91.9% Basic test) (only non-filtered samples) and 24-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 Heterocapsa incorenseus (only 2016 spring samples). The 2015 summer water samples, from both lakes (Mondsee and Irsee) 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 (EC50 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 Irsee, 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 - ISEP and Cebedeu) 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 microcrustacean Daphnia magna. Priority List substances of the WFD and other pollutants discharged in significant quantities were also measured. The analyses were carried out before, during and after the treatment for the tannery effluent 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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Estrogenic-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 48 % to 1 dilution sample to a dilution level of 9.6, using the recombinant yeast bioassay Arxula Yeast Estrogen Screen (A-YES®). For each analysed sample the dilution the 17β-estradiol equivalent concentration (EEQ) was calculated based on the 17β-estradiol (E2) calibration curve. Subsequently the dilution-adjusted EEQ (EEQadjusted) was calculated (EEQadjusted = EEQsample dilution) and the relationship between sample dilution and EEQadjusted was examined by weighted linear regression analysis. Results revealed no differences between EEQadjusted across all dilution levels for the spiked surface water and spiked effluent samples, whereas EEQadjusted 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 water bodies 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.
potential ecological risk of Lake Mondsee. Water and sediment samples from Lake Mondsee are a recreational area in Austria for both bathing and 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 Irsee (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 (SOD and glutathione peroxidase), (iii) neurotransmitter (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 found between both sites in mussels and in lake Mondsee and lake Irsee liver LPO (lower in Lake Irsee). 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.

In Keywords: biochemical markers, fishwater ecosystems, risk assessment, wastewater effluents

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? A. Mezghani, Calula ACCOR; A. PARIS, INERIS; T. Amouroux, Cellulite ARC-IFREMER / RBEBE; M. DALLET, Cellulite ARC - INERIS; S. ANDRES, INERIS / Toxicological Ecotoxicological Assessment of chemical Substances 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 fish, fishing, and economic and social activities depend. The Directive lists four European regions: the Baltic Sea, the North-East Atlantic 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 discharges, emissions and losses of hazardous substances, with ultimate aim of achieving concentrations in the marine environment near background values for naturally occurring substances and close to zero for man-made synthetic substances (background assessment criteria (BAC)). Environmental Assessment Criteria (EAC) are assessment tools intended to represent the control and concentration levels in aquatic organisms expected to occur in marine species. In order to determine which environmental threshold should be used for the implementation of MSFD (EQS, EAC, other threshold), a comparison is done between the methodology 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 Y. Kamada, Chiba Institute of Technology / Department of Creative Engineering Nitrogen and phosphorus are major nutrients to various plants. However, nitrate (NO₃⁻) and phosphate (PO₄³⁻) are notorious in rivers, lakes, by and water reservoirs because they can cause eutrophication in aquatic ecosystems. In Japan, some close 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 high. In this study, concentrations in particulate matters at a time of minimum flow were used to calibrate the passive samplers 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 matters in rivers. The passive samplers 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 matter 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, hydrophobic and hydrophilic 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 of pollution in a shallow hypereutrophic lake T. Augspurger, U.S. Fish and Wildlife Service / Ecological Services; M. Moorman, J. Stanton, U.S. Fish and Wildlife Service Lake Mattamuskeet is a 16,200 ha, shallow (<1 m) 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 SA) 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 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 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.
were compiled from published scientific literature, governmental reports, and the
NORMAN network’s EMPODAT database. The dataset now contains ca. 30,000
supporting the project and the RMs Processing Team of JRC-Geel for technical
were reported 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 reported for each sample. In other cases, the means and ranges were
reported for 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 micropolutants 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 within a framework 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 (27 congeners),
and polycyclic aromatic hydrocarbons (PAHs) were collected in order to determine
critical 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 (carmazepine 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
certifications were determined. The results will be presented and discussed in a
ceremony of marine chemical mixture risk assessment. Acknowledgments
The authors thank the Flemish Government (Flemish Government; AUGE/11016) and from the Ghent University Special Research Fund (01B07512)
acknowledged for the UHPLC-Q-ExactiveSM and the automated SPE equipment,
respectively.

WE249
Development of a rapid screening methods for pathogenic bacteria by using
chemical contaminants in Olympic Marathon swimming courses at Tokyo bay
S. Komeda, Chiba Institute of Technology / Department of Creative Engineering
This study was focused on the 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 and the central government in calculating sum 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, bacteria also carry chemical pollutants. Therefore, these pollutants 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 cotrimoxazol. 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 organics. We also chose caffeine and cotrimoxazol which are 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
C. Liang, Foorin University / Department of Nursing; J. Chen, National Central University / Graduate Institute of Applied Geology

Chronic arsenic (As) exposure continues to be public health problem of major concern worldwide. A geochemistry survey 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

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, fluorene, phenanthrene, 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 CRMs were assessed; their contributions to the uncertainty of the certified values were estimated between 1.7 to 1.4% and 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 CRMs to simulate environmental conditions. This study also contributed to providing a methodology used in the mandatory monitoring of...
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 (Kow = 10 240 000 ml/g; log Kow = 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-EQSs and MAC-EQSs, respectively) can be used to determine 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 obtained from peer-reviewed GLP studies. They were used to assess the water 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 maximum 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 log Kow > 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 bioavailability 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 a method for the implementation 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€3030,000/sup>, 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 classifying sampling sites and comparing them to environmental standards in 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”. - or clear=“all” />
A source apportionment model for regulated chemicals
L.A. Nieuwenhoven, Aktins Ltd; C. Constantino, P. Daldorph, Atkins
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 the 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 CLP/LC5625 contribute to a clean circular economy? The contribution of WEEE plastics recycling
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Most 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
Plastics are an indispensable 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 management stakeholders and users 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 recycling industry. Also, economic considerations played a major 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 recylcate 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. Jurbeg, Dow AgroSciences LLC
Introduction: There are different classification and labelling (C&L) systems and responsibilities bodies for chemicals across the globe. Many jurisdictions have adopted the United Nations Globally Harmonised System (GHS), either in whole or in part. 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. Beside, respiratory sensitisation (100% compared to the dossier single 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 assessment (P)
C. Terry, Dow AgroSciences LLC; G. Meregalli, Dow Agrosciences; D. Jurbeg, Dow AgroSciences LLC
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 organisms (e.g., fish) than the concentration in the ambient water column. MeHg is known to exhibit various toxicities such as neurotoxicity, cytotoxicity, and development 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 used. The 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) were 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 were up-regulated with concentration-dependency of MeHg. In the case of GST, 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, 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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Nanoparticles are part of everyday life. Nanoparticle (NP) concentrations in natural environment are not yet known but releases during their life cycle are to be considered. NP harmful effects have already been demonstrated for freshwater and marine species but their potential toxicity at more realistic low exposure concentration is still not well studied. Cerium dioxide nanoparticles (nCeO2) 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 nCeO2 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. The 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 multi stress 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 standardised and commercial NPs but also to mimick 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 nCeO2. 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 koreanae
J. Park, J. Lee, Sungkyunkwan University

2.2'-4',4'-tetrabromodiphenyl ether (BDE-47) is widely dispersed endocrine disrupting compound in the aquatic environment. Due to its disruptive effect on marine organisms and insufficient database on toxicity, 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 koreanae. In B. koreanae, 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-α, -γ, 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 activity have not been observed at 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. Seift, J. Swinn, University of Portsmouth; A. Ford, University of Portsmouth / Biological Sciences

Numerous active pharmaceutical ingredients (APIs), which primarily enter the environment following their introduction into effluent systems, 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 (Fix), a type of selective serotonin reuptake inhibitor (SSRI), is still one of the most popular antidepressants prescribed. Serotonin is one of the chemicals that controls mood. The SSRI exposure is expected to alter emotional based behaviours in humans, serotonin is involved in multiple biological pathways in invertebrates. Despite the main role of Fix being to concentrate serotonin in synaptic crests through blocking of serotonin reuptake proteins (SERT), some pharmacologists have described them as ‘not so’ selective reuptake inhibitors for the broader receptor membrane. SSRI exposure is known to effect monoamine synthesis and neuronal 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 gene expression development markers in the crustacean Echinogammarus marinus and Palearmnowetes 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 released throughout the haemocoel. Experiments are ongoing to quantitatively link changes in behaviour to Fix exposure and changes to expression of neurotransmitters and neural biomarkers development in P. vulgaris.

Keywords: Fluoxetine, SSRIs, 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 marine taxa, it is largely 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-masculisation, 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-masculisation 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-masculisation in field populations of crustaceans.

TH006 Development of biomarker genes for endocrine disruption assessment in the non-model crustacean species Gammarus fossarum

Despite the importance of crustaceans in aquatic ecosystems, 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 aquaculture/taxonomy. In this project we have developed a multi-disciplinary 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 potentially important for ED assessment in crustacean taxa have not been 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, genes of the hormone metabolism and the 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 responses and embryo development in benthic amphipod crustaceans in the assessment of sediment toxicity
N. Berezina, Russian Academy of Sciences / Zoological Institute; K.K. Lehtonen, A. Alvo, 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, antioxidant defence responses and embryo development in the benthic amphipod Gamlinoidea 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 toxicity testing (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 1:3 to 1:1024. The lowest dilution contained 6064 µg kg⁻¹ of polyaromatic hydrocarbons, 2030 µg kg⁻¹ of tributyltin, 2862 µg kg⁻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 (%) of G. fasciatus was evaluated in a 10 toxicity test, showing 100% mortality in the toxic peak of the reference sediment or in 1:256 and 1:1024 dilutions. Next, after a 28 days 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 reference treatment (<5%). In the 1:256 dilution the total number of aberrations was lower (31%) compared to the reference, while embryo frequencies 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 edosyne-related genes in Chironomus riparius after exposure to reined water and its fortification with triclosan and carbamazepine
O. Herrero, UNED / Grupo de Biología y Toxcológia Ambiental; R. Planelló, UNED / Grupo de Biología y Toxcológia; E.M. Beltrán, INIA - National Institute for Agricultural and Food Research and Technology / Environmental; C. Fernández, INIA National Institute for Agricultural and Food Research and Technology / Department of Environmental Chemistry, INIA - National Institute for Agricultural and Food Research and Technology / Environment 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 be 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 reproductive 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 edosyne-related gene expression (vitellogenin and edosyne receptor) of C. riparius by using flow-through system 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 overlying water were also made to assess the sediment distribution of contaminants. RW contained a number of recalcitrant organic compounds and this fortification approach should simulate a simple mixture exposure under laboratory conditions that could be useful to understand relationships between these apical endpoints and the transcriptional variations of the transcription factors as well as functional development in Chironomus riparius. 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
J. Rouxel, R. Sussarellu, IFREMER, F. Alchu, IFREMER / Laboratory of Ecotoxicology
The contamination of aquatic environment by pesticides and the associated toxic effects on the marine organisms are widely recognized. Many studies described the induced effects of copper and diuron during 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 Ca²⁺-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-fecundation) were exposed to 4 nominal concentrations of copper (0.10, 1.00, 10.00, and 20.00 µg L⁻¹ Cu²⁺) and diuron (0.01, 0.05, 0.10 and 0.50 µg 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 Ca²⁺-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 µL⁻¹ and 0.05 µg L⁻¹. Preliminary on ion pumps show a good sensibility and reproducibility of the assay. The measurement of Na/K-ATPase and Ca²⁺-ATPase activities in exposed embryos are ongoing.

TH100
Suitability 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), acetylcholinesterase (AChE) and glutathione 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.

TH101
Phenotypic defects in Gammarus fossarum exposed to fenoxycarb as embryo
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The use of embrios 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 hatchling success as the endpoint of interest. In the present study, embryos of the amphibid Gammarus fossarum were directly exposed throughout the entire embryo cycle to increasing fenoxycarb concentration (0.0, 0.5, 5 and 50 µg 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) developmental delay. Fenoxycarb caused a 5 and 50 µg L⁻¹ delayed embryo hatching. Eye pigmentation was impaired in the 5 µg L⁻¹ exposed group. Gnathopod centroid size was increased in the 5 µg L⁻¹ exposed group while antenna centroid size was reduced in the 50 µg L⁻¹ exposed group. Additionally, we detected digestive tract tissue damages in the 50 µg L⁻¹ exposed group. This gammarid embryo lethality assay provides a model 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 hawaiiensis
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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 surface. Sediment testing with P. hawaiiensis is used to evaluate the toxicity of different sediments. Since 2011, the project has been ongoing and results show that almost all of the samples analyzed were toxic with higher values for the 1:1 treatment. In order to improve the quality of the analysis, we have developed a new protocol using a flow-through system 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 daphnids in fresh water was developed to provide homogeneous algae suspension which is necessary for 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 hydrostatically stable test substance was carried out. The stability of the test item ensures the same test item exposure in both flow-through and batch systems (semi-static). 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.

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 physico-chemical characteristics of the sediment. Therefore, it is ecologically relevant to examine the influence of contaminants on bioturbation by organisms, such as T. tubifex. 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 days exposures to clean or Cu-spiked sediment (6 Cu concentrations). Experiment A: examination of the effect on growth 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 may be harmful to Daphnia magna. The problem of 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 used in this study was improved by Rodts and Rombouts' method more useful for ecotoxicity 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
N.N. Pedersen, Copenhagen University / PLEN
Pesticides are widely and intensively 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, larva, 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. 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-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. In this study we 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 EC50 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 EC50 for both treatment were 190 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 EC50 for the beetles were 190 ng/mL for the pupae 112 ng/mL and for the larvae it was 420 ng/mL. The EC50 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 and overall the highest EC50 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 larva-stage.
morphology.

B. Caglerdi, The University of Melbourne / School of Biosciences; V.J. Pettigrove, The University of Melbourne / Zoology; S.M. Long, CAPIM / School of Biosciences; A. Hoffmann, The University of Melbourne / School of Biosciences 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 unimpacted 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. Deformities were 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 influencing 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 teppei. 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, accounting for the confounding effects of mortality, which has 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. Fluctuating asymmetry is a frequently 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 Gy/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 alpha and beta sites. Environmental 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 ecotoxicology of some sub-lethal 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, on D. tigrina, which were performed at three 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 LC50 for phenanthrene (8.901 μg L⁻¹), since no results were available 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 LC50. 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

T. Shashkova, Siberian Federal University / Department of Ecology and Environmental Sciences; Y.S. Grigoryev, Siberian Federal University / Department of Ecology and Environmental Study

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 (algae 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 noise 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 better 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, J.M. 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 published laboratory procedure for bioassaying the freshwater pond snail, Lymnaea stagnalis. This 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 existing embryo bioassay methods proposed within regulatory guidelines. Key words: biochar particles, chronic reproduction study follows the OECD 211 test guideline, three enantiomers, but also to differing biotransformation rates and toxicity in organisms. Even though the physicochemical properties of enantiomers are the same, apart from the rotation of polarized light, their biological activity often varies considerably. This may be due to different mechanisms of action or differences in the rate and extent of metabolism. The marine environment can be split into three distinct zones: estuarine, coastal and open ocean. The study followed the then draft OECD test guideline for marine risk assessment which involves acute and chronic tests with earthworms was also performed and changes in body mass recorded. Overall, the LC50 for both phenanthrene and cationic surfactant (SDS) was 50% lower than that obtained in acute toxicological tests conducted previously. The present study concludes that exposure to these microbial insecticides based on the bacterial pathogen Microbacterium fermentans (VectoBac® 12AS - VB) and on the fungal pathogen Chrysonilia fischeri. microbial insecticides based on the bacterial pathogen Microbacterium fermentans (VectoBac® 12AS - VB) and on the fungal pathogen Chrysonilia fischeri.
RENLYS® 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 by 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 early feeding or embryo survival. In all three scenarios a concentation 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 reproduction was not attributed to radionuclide contamination and were driven by a biotic factor in the environment.

### TH022

A comparative assessment of chronic toxicity between freshwater (Daphnia magna) and marine (Tisbe battaligai) test species

F. Simao, Aveiro University & CESAM; C. Gravato, Universidade de Aveiro / School of Biosciences; Y.S. Grigorev, Siberian Federal University / Department of Biology and CESAM; J. Pestana, CESAM & University of Aveiro / Biology

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 and bacteria and on their toxins have been shown to be 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 fungal pathogens *Bacillus thuringiensis* var. *israelensis* (VectoBac® 12AS - VB) and on the fungal pathogen *Beauveria bassiana* (Naturalis-L - NL). Organisal endpoints such as survival, larval growth and emergence were used. Effects on the energy reserves were also measured. Acute tests presented a 48 h LC50 of 1.85 µG/L for VB and of 34.77 µG/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 sub-lethal exposure to both VB and NL. 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.

### TH023

Validation of a short-term, in vivo screening method for detecting chemicals with juvenile hormone activity using adult Daphnia magna (HJSSST)

M. Fox, Scymaris Ltd / Ecotoxicology

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’ persist 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 changing environmental conditions, 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 development/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.

### TH024

Toxicity of microbial insecticides towards the non-target species Chironomus riparius

M.D. Bordalo, University of Aveiro; C. Gravato, University of Aveiro / Department of Biology and CESAM; D. Campos, University of Aveiro / Biology

RENLYS® 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 by 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 early feeding 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 reproduction was not attributed to radionuclide contamination and were driven by a biotic factor in the environment.

### TH025

Influence of biochar particle size 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 biochar 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. Anning for ecologically representative approach, a 28-days moscos experiment was conducted looking into survival of *E. andrei* and micro-organisms’ feeding activity using the bait lamina method. After 28 days, soil leaches from the moscoses were collected to assess effects on *D. magna* growth and reproduction and on *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 moscoses, 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 omega-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 synthesized as a mixture of enantiomers, 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 differences in their effect on non-target organisms. In this study, the relevance of enantioselectivity of chiral pesticides in

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ecotoxicology and mixture toxicity was explored. Azole pesticides such as imazalil have been shown 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 seemed to be related. The goals of this study were to compare the 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 effects 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 components 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.

D. similis and D. magna are the dominant species in freshwater indoor microcosms. They also varied in terms of their content. Aqueous solutions of mancozeb proved to be more sensitive towards chronic exposure to the soil ecosystem and are sensitive to soil contaminants. The aim of this study was to develop a new and highly sensitive micro-impedance sensor system (MSS) 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 (EC50) 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 EC50 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=.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 functionality of these organisms. Thus, the introduction of functional endpoints is essential.

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 established. Methods for their use are described in protocols. 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 the cellular components of the hemolymph with coelomic fluid (coelomocytes). 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 group 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 cocking, 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 Hemaocolor® Rapid staining of blood smear. Approximately 50 cells per sample were analyzed. During the microscopic evaluation, each cells as basophilic and acidophilic leucocytes, erythrocytes, 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 chloramphenicol (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 assessment of the effects of chemicals on soil organisms, but further studies should be conducted.

TH030 The comparison of the sensitivity of the collombolan, 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 present 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 collombolan, Folsomia candida. Both earthworms and collombolans are integral parts of the soil ecosystem and are sensitive to pollution. The experiments were performed in compliance with OECD Guidelines No. 222 and 230. Three insecticides (acetamiprid, indoxacarb, and alachlorpyrtrim), 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 EC50, EC20, and NOEC were calculated using the ToxRaT Professional statistical computer software. Available literature data indicate that collombolans 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 collombolan and the earthworm reproduction, which did not depend on the type of the pesticide used in the study. It was noticed that the collombolans were more sensitive to the pesticides containing acetamiprid, metamitron, and s-metolachlor than the earthworms. No significant effect of metamitron on the earthworm reproduction was observed. The pesticides which include indoxacarb, alachlorpyrtrim, mesotrione, dodine, dithianon, and thiophanate methyl strongly impacted the earthworms. There was no significant effect of alachlorpyrtrim 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 of pesticides on the collombolans is different to that of the earthworms. The sensitivity of a pest to a pesticide 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 Daphnia magna after exposure to reclaimed water.

M. Aquilino, UNED; J. Martinez-Gutiarte, UNED / Fisica Matematica y de...
Fluidos; P. Sanchez-Arguelles, 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 sensitivity 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 reared 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 EBR, Rsr 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 (methyiparaben, IP60 and fluoxetine). Additional methods for ecotoxicology and mixture toxicity were discussed herein. This work has been funded by the Spanish Ministry of Economy, Industry and Competitiveness through CTM2013-44986-R project. M. Aquilino is the receiver of a predoctoral contract Ministry of Economy, Industry and Competitiveness (BES-2013-064041).

TH032 Development and test of a new micro-impedance sensor system for water quality monitoring
A. Gerhardt, J. Mahlke, LimCo International GmbH; S. Wirtzsohn, O. Hamburger, J. Reuter, HTWG 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 subterranean 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 bioossenes. 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. Early culture and handling additionally opt for E. serrulatus as new indicator in biomonitors.

TH033 Ecotoxological assessment of phosphate recycles from sewage sludges
N. Rastetter, LimCo International GmbH; K. Rothapall, University of Constance; A. Gerhardt, LimCo International GmbH 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 bioreos, 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-recycles (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 crustacean Gammarus fossarum by mortality and behavior (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 and more than 95% of the PRs at the highest tested concentrations (5-10 g DM -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 -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)
J. Baumann, Eurofins Agroscience Ecotox GmbH / Aquatic Toxicology; H.M. Maes, M. Wucherer, J. Wiewel, G. Gionis, Eurofins Agroscience Ecotox GmbH / Aquatic Ecotoxicology; M. Candolfi, Eurofins Agroscience Ecotox GmbH / Aquatic Toxicology; C. Halfer, Eurofins Agroscience Ecotox GmbH / Aquatic Ecotoxicology 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 202 (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 213 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
M. Pipal, Masaryk University, Faculty of Science, RECEOTOX / Research centre for toxic compounds in the environment RECEOTOX; T. Prochazkova, Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University / Research centre for toxic compounds in the environment; J. Priebojová, Masaryk University, Faculty of Science, RECEOTOX / Research centre for toxic compounds in the environment RECEOTOX; E. Congiu, Masaryk University / Research centre for toxic compounds in the environment RECEOTOX; M. Smutna, Masaryk University / RECEOTOX; K. Hilsherova, Masaryk University, Faculty of Science, RECEOTOX / Faculty of Science, RECEOTOX Cyanobacteria produce and release to the aquatic environment various types of bioactive compounds, which may affect the development of fish or other aquatic 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 polychlorinated biphenyls. 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 vitro 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 that compounds are acting in the same or 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 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 role as a component of real-world environmental media, several basic studies have 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. Gaugetic Mystus, Mystus cavasius, is a small-sized freshwater fish that is available in high population density 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, 8 µg/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 5.6±0.59 to 5.5±0.53 mmol L⁻¹ and in treatment from 6.1±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 7.8±0.29 g.dL⁻¹ in treatments. In male it ranged from 9.0±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 in an influence of cypermethrine exposure. In female, monthly RBC levels (±10 mm-3; Mean ±SE) ranged from 0.59±0.06 to 1.18±0.05 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 0.36±0.03 to 1.88±0.36 in male. WBC counts elevated in most of the female and male were 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 naturally occurring polycyclic aromatic hydrocarbons (PAHs) in sediments from the oil sands area of Alberta that naturally contain 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 by causing 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-deethylxiation (EROD) and benzoyloxy-4-trifluoromethylcoumarin-O-debenzyloxylolation (BFCOD), were used as markers for trout hepatic CYP1A, and CYP3A activities, respectively. Hepatic microsomes were exposed to six concentrations of each pharmaceuticals, including environmental relevant concentration (0.01 to 100 µM), test for cypermethrin exposure in aquatic animals and calls for the limited and cautious use of cypermethrin.

TH040 New test for cypermethrin exposure in aquatic animals and calls for the limited and cautious use of cypermethrin.
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 approx. 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 80-109% 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.

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 chemicals (i.e. biocides, herbicides 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-picrocarmine-acid fuchsin (van Gieson, collagen differentiation), iron hematoxylin-periodic acid-Schiff reagent-alcan blue (AB-PAS), detection of polysaccharides, mucosubstances and mucins in tissues), iron hematoxylin-acid fuchsin-phosphomolybdic acid-methyl blue (Mason’s trichrome, connective tissue differentiation), silver nitrate solution (AgNORs, silver-binding nuclear 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 developed epidermis and also quaternary aminoacids changes in 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 it 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.

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 well studied 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 liver homogenate samples were collected with a bursa fenestrated needle, 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 (dichlofenac, 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 dichlofenac 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 to 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.

Developing Screening Assays in Zebrafish

F. Smrt, Uniop Biometrace Inc

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. We present results for assay development using VAST BioImager.

Toxicological assessment of novel potential fungicide in vitro cytotoxic effects in zebrafish (Danio rerio) hepatocytes

M. Mareszews Romoowko, Faculty of Fisheries and Protection of Waters; G. Zamaratskaya, Swedish University of Agricultural Sciences; V. Burkina, University of South Bohemia in Ceske Budejovice / Faculty of Fisheries; C. Sullivan, K. Shires, C. Lavalle, Q. Rochfort, Environment Education, Youth and Sports of the Czech Republic, projects CENAKVA (No. TH036 LM2015051).

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Cytotoxic effects of a novel potential pesticide (MgPhenHesp2) on zebrafish (Danio rerio) hepatocyte cell line (ZF-L). A clear impact on ZF-L cell population was noted 24 h after MgPhenHesp2 exposure evaluated by cell count/number and cell/counts density (CH2O 7.8%), excited, homogenous 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 observed. However, MgPhenHesp2 concentrations (0.1µg l-1 to 1µg 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 understanding and accurate assessment. Additional comprehensive, in vitro assays 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(Hsp); 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 development 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)). By 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 activity of AhR-regulated enzymes increased 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 (HSP90, 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 altered 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 a 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(Hsp); effects on the cells are extremely important to point out integration between in vitro and in vivo responses.

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 systems using QPCR. Our results show that mainly the enzymes at the beginning of the steroidogenesis pathway are maternally transferred. This suggests that the role of maternal transferred genes is in determining the earliest stages of zebrafish development before the embryo’s genome is activated around 3hp (hours post fertilisation). Further, distinct transcriptional patterns of estrogen receptors are noticed during development. Ersl is abundantly transcribed from the time of embryonic genome activation and transcription of er2b increases during the formation of the immature gonad. High transcript levels of er2a due to maternal transfer are observed during later differentiation. Moreover, 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 ontogeny of steroid hormone and functional insights underlying aversive effects of exposures to mianserin. Analysis of the relationship between effects in the locomotor activity and the prevalence of autism spectrum disorder has recently been increased in Asia, social interaction, impaired communication, and repetitive behavior. The still unclear. Transcriptome analysis would be helpful to understand the underlying mechanism of VPA exposure. In addition, 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 transcriptome 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 affects a large number of genes that are involved in the development of the various brain regions. Depressed motor activity in larvae is a hallmark of early life stages of the 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. 

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 research 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

S. Lee, Seoul National University / System Toxicology Center; H. Chun, S. Yoon, W. Kim, Korea Institute of Toxicology, KIT / System Toxicology Center

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 transcriptome 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 affects a large number of genes that are involved in the development of the various brain regions. Depressed motor activity in larvae is a hallmark of early life stages of the 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 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. Also, chemicals are extensively used worldwide. Their potential as developmental toxicants is highly analyzable, and their effects are also linked to neurodevelopmental 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: mycobutanol, penceonazole, dieldrin, methoxychlor, difenconazole and triclocar. Firstly, we carried out developmental toxicity assays. For this purpose, we selected wild type embryos at hatch from 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. This would allow us to assess developmental and neurotoxicity 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

Dario roiro

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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, we 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, 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 are detected in the aquatic environment. Even low concentrations are potentially of concern due to their design towards specific drug targets, which are evolutionarily conserved in aquatic organisms, especially in fish. β-adrenergic receptor antagonists (β-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 β-blockers have been observed in different fish species and can be related to the pharmacological modulation. β-blockers are white 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 biobarrier more easily than the corresponding ionic species. This difference in uptake might result in an enhanced activity of β-blockers at neutral pH. Moreover, the influence of pH on toxicity of four β-blockers in zebrafish embryos. The zebrafish Danio rerio is a popular model organism for fish and its 96th 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, Labelatol 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 Vertebrate 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, Labelatol and Propranolol on heartbeat and swimming activity. All β-blockers showed an increased 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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Microcystis aeruginosa is one of the primary ecotoxicological testing guidelines. Gene transcription of various chemicals. The Fish Early Life-Stage (FELS) Toxicity Test (OECD TG 210) is one of the primary ecotoxicological testing guidelines. Gene transcription of various chemicals. The Fish Early Life-Stage (FELS) Toxicity Test (OECD TG 210) is one of the primary ecotoxicological testing guidelines. Gene transcription (BNF), polychlorinated biphenyl 126 (PCB126), benzo[α]pyren (BaP)). By using new insight into the regulation of the AhR-pathway and show that it is very increased activity. Experiments with BaP are in progress. Our results provide a mechanisms of cellular responses. Thus, further studies on MgPhen(Hesp)2 effects are underway in intestine and liver samples. Changes in expression of target genes by real time quantitative polymerase chain reaction (Q-PCR) for the following genes: catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione-S-transferase (GST), cytochrome P450 (CYP1A), protein phosphatase (PPP), 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</strong>: Sublethal dietary exposure, Microcystis aeruginosa, microcystin-LR, Zebrafish gut microbiota.

TH053 Mixture-specific gene expression in zebrafish (Danio rerio) embryos exposed to perfluorocarboxlate sulfonate, perfluorohexanoic acid and 3,3',4,4',5-pentachlorobiphenyli

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Perfluorocarboxlate sulfonate (PFAS) and Pentachlorobiphenyli (PCB126) are dominant organics pollutants withdrawn from the market but remaining of high concern because of their environmental persistence, bioaccumulation and toxic properties. Additionally, the amphiomorphic 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 activations 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 (ahr, cyp1a), oxidative stress (gsta1, tp53), lipid metabolism (accu2, osbpl4), and epigenetic mechanisms (dnmt1, dnmt3a) 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 mixture. However, after 72 and 96 h, cyp1a expression was significantly lower when embryos were co-exposed to PCB126+PFOS+PFHxA. Significant
upregulation of gxlα was observed after exposure to PCB126+PFHxA and to the ternary mixture at 30 hpf and 48 hpf. Besides, embryos appeared more sensitive to the ternary mixture at 78 hpf: acua2 and ovphl1 were significantly downregulated; dmnt1 was significantly upregulated. In conclusion, the present results show that PFOS and PFHxA are modifying PCB126-induced gene expression in zebrafish embryos, suggesting that these chemicals are able to modulate their toxicity. However, most toxicological investigations are required to gain a better understanding of the mechanisms leading to a decrease of cvpla induction in presence of the three chemicals.

TI054
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 estrogen-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 tests, as it is needed to confirm the suspected liver toxicity. Therefore, an intimate understanding of the interplay between primary estrogen-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. Hepatotoxins with different modes of action, namely acetaminophen, isoniazid, valproate and dimethyl-O-creosol (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) transcriptional analyses (RNA-seq) of genes involved in energy metabolism and (5) rpsometric measurements of the energy budget to assess liver functional toxicity.

TI055
Coexpression effects of 17α-ethinylestradiol in the presence of micro- and nanoplastic on embryo development and larval behavior of Danio rerio M. Grabbach, RWTH Aachen University / Department of Ecosystem Analysis; q. Chabot, RWTH Aachen University / Department of Ecosystem Analysis

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 microplastics (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 analysis of the ‘trojan horse effect’ reagarding to the changes of the behavior of Danio rerio larvae. The coexpression effects of 17α-ethinylestradiol (EE2) with a concentration of 2 and 20 microgram/L and MP with a concentration of 1 milligram/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 of swimming distance and distance between zebrafish larvae could be detected. The analysis of the coexpression 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. Glap, alphal-1-antitribulin and zfrg as two genes for neurodevelopment monitoring and zfrh and zfrg 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 combiniative analysis of the effects between EDCs and MP. The co-exposure between both components influence and change the bioavailability and toxic effects of exposure to one compound, 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 influence the processes on other ecosystem levels.

TI056
Effects of bisphenol SIP on HPG axis and reproduction in zebrafish (Danio rerio) j. Lee, Yongin University; B. Kim, Yongin University / Department of Environmental Health; K. Ji, Yongin University

4-hydroxyphenyl 4-isoproxyphenylsulfone (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 higher 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 hypothalamic-pituitary-gonadal (HPG) axis and reproduction were investigated using adult zebrafish. At dosages of 50, 500, and 5000 μg/L, the hormone (luteinizing hormone, LH) responses were respectively observed at 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 investigated. 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 17β-estradiol (E2)/testosterone (T) ratio was observed at ≥5 μg/L BPSIP along with up-regulation of cvpla transcriptions. Concentrations of plasma T were significantly increased in females exposed to 50 μg/L BPSIP along with up-regulation of cvpl 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-2015R1D1A1A01056268).

TH057
Response of zebrafish and Japanese medaka to imidacloprid: a comparative study c. vignet, Eawag / UTOX; K. Lajoie, EPOC University of Bordeaux; M. Maisano, University of Messina / Department of Biological and Environmental Sciences; H. Mottaz, Eawag / UTOX; C. Clerandeau, EPOC University of Bordeaux; T. Cappello, University of Messina / Biological and Environmental Sciences; K. Schirmer, Eawag / Environmental Toxicology; J. Cachot, EPOC University of Bordeaux

Neonicotinoids are among the most produced and used insecticides and are frequently found in freshwater with concentrations ranging from ng to μg/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 μg/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 μg/L imidacloprid exposures was observed for hatch in both species. Except for the 20 μg/L exposure level, imidacloprid led to hypovitility in both species. A striking difference was found in morphology: a high number adult deformities were noticed for medaka whereas almost none were found in zebrafish. Yolk and bone oedemas started to be noticed at 20 μg/L after hatch, jaw deformity and lordosis/scoliosis increased with concentration level. An increase of haemorrhage was found at the highest concentration (2000 μg/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 A.C. Brooks, Cambridge Environmental Assessments / Regulatory Ecotoxicology; S. Rackman, Cambridge Environmental Assessments / Toxicology; K. Ott, BASF SE / Ecotoxicology; D. Sprenger, BASF SE

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 (EFSAs 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 1 risk assessment for waterbodies, the lowest available adverse effect level (LOAEL) 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 EFSAs (2009), in terms of which type 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 (EFSAs supporting publication 2015: EN-924. 62 pp.) that ecologically relevant reproductive endpoints should be assessed and agreed during action plans for establishing EU level 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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Ecotoxicology - Tieral Вертебраты Expert Team
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 toxicogenic cyanobacterium Microcystis aeruginosa produces different negative effects on the survival and reproduction of Daphnia curvirostris
F. Martinez-Jerónimo, Escuela Nacional de Ciencias Biológicas-I.P.N. / Laboratory of Experimental Hydrobiology; L.L. González-Trujillo, Escuela Nacional de Ciencias Biológicas-I.P.N. / Departamento de Biología Utopofía. 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 (M) synthesizes 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 toxicogenic 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 toxicogen strain M. aeruginosa NUV-5 was grown in 2-8 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 crude extract of cyanobacterium body were tested in the D. curvirostris under 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 LC50 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 μg L-1, respectively, in the acute toxicity assays MCs concentration ranged from 1.5 to 2.3 μg L-1. Survival rates to 72 h. - Survivors from 24 h to 72 h. Survivors to 24 h to 72 h. and for 72 h. S. mixtus exposed to the MCs produced by A. aquaticus under each condition was determined. The 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 (Rs) and the intrinsic growth rate (r). The size of juveniles released in the first 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 size 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 concentrations. In the cladoceran S. mixtus, the analysis of the metabolomic profile in response to the exposure to glyphosate, FAENAs® 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) in 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 LC50 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 (Rs) 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 size 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 concentrations. In the cladoceran S. mixtus, the analysis of the metabolomic profile in response to the exposure to glyphosate, FAENAs®, 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 of long term exposure to imidacloprid on Ansellus 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 Ansellus 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 fish, while the imidacloprid treatments were triplicated. Each replicate consisted of a 5L bucket (Figure 1) filled with 20 specimens 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 substrate. 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 5 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.
effects in the marine amphipod, Echinogammarus marinus

N. Fuller, University of Portsmouth / Biology; J.T. Smith, University of Portsmouth / School of Earth and Environmental Sciences; A. Ford, University of Portsmouth / Biological Sciences

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 radioactivity 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. Ample evidence is available to assess male reproductive ability at a population level 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, 0.1, 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 observed data suggest 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 ectoparasitc salmon lice (Lepeophtheirus salmonis) has gradually become a major problem. A commonly used pesticide against this crustacean is diflubenzuron (DB15), which acts as a chitin synthesis inhibitor and thereby interfere with the moultng stages during sea lice development. However, DBF 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 demonstrated that shrimp exposed to DBF through fish feed have a reduced survival (ca. 60%) compared to control, in both the larval and the adult stages. Moreover, the effects of DBF 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 presents challenges in selecting 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 schedules and different degrees of exposure for the shrimp populations. The purpose of the model is to predict effects of DBF exposure on population-level endpoints such as larval survival and abundance, and age structure, and to assess the risk of population decline below threshold abundances.

TH065 Assessing the spatial distribution of the gastropod Olivella seministra 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 seministra on the Ecuadorian coast (city of Maníta) is influenced by urban discharges. Therefore, contamination could determine habitat areas for snails. Field studies represent studies that aim at understanding the ability of individuals to detect local contamination and avoid inhabiting contaminated sediments. Secondly, the ability of snails to recognize contaminated sediments under recovery was studied. Sediment samples were taken in five points (El Murcielago beach - reference point, El Puerto, La Poza, Rio Burro, Los Esteros, and Rio Muerto) and evaluated for avoidance behavior and recolonization trials. Two experiments 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 confining the population of distribution and isolate populations. Keywords: Olivella seministra, 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 7×105 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, although, 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, and 32 mg L-1 DB15 (96 h at 25°C, continuous illumination of 120 μmol m-2 s-1). Cladoceran neones were exposed to 150, 200, 250, 300, and 350 mg L-1 DB15 (48 h at 22°C, light intensity of 120 μmol 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 μmol m-2 s-1 and 16:8 h photoperiod). A. falcatus was the most sensitive to DB15 (EC50 = 28.97 mg L-1) of all the tested organisms. The LC50 values for D. magna and C. rigaudi were 223.8 and 29.5 mg L-1, respectively. The zebrafish embryos were more sensitive to the non-environmentally relevant values. The hatching percentage of D. rerio larvae decreased significantly as DB15 dye concentrations increased. Lethal effects, according to OECD guideline 215 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 warn 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. Further, they may only be present in the environment at low levels. 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 new chemical testing methodology needs to be developed for these substances, 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 mediators 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 F.M. Bakker, Eurofins-Mitox; J. Roig, b. dehelean, R. Delhem, A. Grove, F. Faraji, Eurofins-Mitox Consultants; M. Schouten, Mitox Trial Management BV; G. Pennards, W. Heitsmans, A. van Eek, I. da Silva, C. van Houdt, Eurofins-Mitox Consultants 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, EC₅₀-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 simple 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 assignments 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 EC₅₀ - derived SSD’s. Using HC₅-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 O. Jakobs, Rifcon GmbH / Population Modelling and Statistics; T. Wittwer, Rifcon GmbH; L. Ibraim, Rifcon GmbH / Regulatory Affairs Environmental Fate Modelling; F. Bastiansen, J. Ludwigs, Rifcon GmbH In environmental risk assessments (ERA) for plant protection products (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 ecological recovery after pesticide application for population modelling studies using stochastic models (such as BMs), 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 four 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 A. Toschki, J. Jollers, N. Willius, gaia Research Institute for Ecosystem Analysis and Assessment; M. Hammers-Wirtz, Research Institute gaia 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 Nonmosibl sponsored by the European Federation for Microbiological Research (EFMB) the effects of silver nanoparticles on the soil mesofauna are examined in terrestrial model ecosystems (TME). To set up 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 were labelled as the nature of the location to the new setting in the laboratory. Five different concentrations of silver nanoparticles (AgPure) as well as four concentrations of a toxic reference (AgNO₃) 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 by using the standard procedure in a McFadyen extractor according to the ISO 23611-2 guideline. In this presentation the dose response related results for oribatid mites and collemboans 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 A.O. Ogunbemi, University of Koblenz-Landau; K. Kerngott, K. Altendorfer, S. Kurtz, University of Koblenz-Landau / Institute for Environmental Sciences; G.E. Schaudemann, University Koblenz-Landau; C. Bruehl, University of Landau, Institute for Environmental Sciences / Institute for Environmental Sciences; C. van Gestel, VU University Amsterdam / Ecological Science; M. Kurtz, University of Koblenz-Landau / Institute for Environmental Sciences 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 expected to potentially produce different effects in the population. 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 extensive 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 AgNO₃/L respectively. The higher sensitivity of the reproductive-active 22-day old 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. Duroo, 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 SimpleTreat 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 SimpleTreat for an industrial EU manufacturing treating on-site the effluents through 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.
The modeling domain covers South Korea where the spatial resolution in air is independent of chemical properties. The rate constants of photodegradation and BghiP were of the second largest magnitude. The magnitude of these rate constants were estimated by assessing the changes in the fugacity ratios (FR) between the pairs of air and soil, air and water, water and soil, 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) when CC was assumed to begin in 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 (FRwater/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. FRsediment tends to decrease (~70%) except for the PAHs of 5 and 6 rings and 1,2,3,4,6,7,8-HgCCDD. For all the compounds, FRsediment tends to decrease to some extent (~78.8%) while FRgas 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., FRwater/air and FRsediment) 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.

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 2011-2013 and 2014-2015 and the mean concentration changes with respect to the indoor-outdoor temperature difference was compared between measured (-3.9%°C) and simulated (-4.4%°C) 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, 2041-2070 (F1), 2071-2100 (F2) 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 increases by 7% (t-test, p<0.05). The maximum annual average concentration for the F3 period increases by up to 28% 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.

A Double-Diffusion Model to Quantify the Adsorption Effects of Outdoor 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 test chemicals were assumed to diffuse from mm-thick vinyl flooring, and the indoor surfaces considered were gyspum 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 was maintained for tetradecane after 150 days. In addition, 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 source.

TH078
Environmental exposure scenario for reagents used in vitro diagnostics
V. Stark-Rogel, F. Hoffmann-La Roche Ltd.; K. Urmann, A. Haener, Arcadis Schweiz LTD; F. Hoffmann-La Roche Ltd.
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 concentrations 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 concentrations 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. The resulting generic diagnostic waste scenario demonstrates that in situ 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 Umweltchemen Ökotox, 2016, 2, p. 44-50

TH079
Integrated external and internal exposure to chemicals: the INTEGRA computational platform
D. Kong, Chevron Energy Technology Company; W.R. Gala, Chevron Energy Technology Company; R. Arnold, Chevron Energy Technology Company; V.R. Gal, Chevron Energy Technology Company; HES; G. Douglas, J. Hardenstein, S. Rouhani, NewFields Environmental Forensics; E. Littman, NewFields Environmental Forensics Practice, LLC
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 polyaromatic 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 < 0°C. The technical basis for these requirements is not well defined (NTFailing 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 subjected to incremental intervals up to 60 days. Statistical analysis of the data demonstrated no degradation of PAHs in the

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 vinyl flooring being dominant among them. The overall life cycle assessment of vinyl flooring manufacturing showed that environmental contribution to overall aggregate exposure of 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 m² and air exchange rate of 0.5 h⁻¹) characterized by DEHP gaseous emissions of 200 μg/h (vinyl flooring and other plastic materials), the concentrations of DEHP in the gaseous, particulate and dust phase are equal to 1.5 μg/m³, 21 μg/m³ 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 vaccines administered and in adipose tissue increases. A recent study (Zak et al. 2011) 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 SOx-OH-2MEHP 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 the 19th century and in addition to traditional industries such as foundry, steel, mechanical 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 (SRN) 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 vegetation cover; 2) identify spatial and temporal fingerprints of the contamination process; 3) test different weather scenarios; 4) compare the predictions with the regional fingerprint 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
R. Arnold, Chevron Energy Technology Company / Environmental Risk Assessment; D. Kong, Chevron Energy Technology Company; W.R. Gal, Chevron Energy Technology Company / HES; G. Douglas, J. Hardenstein, S. Rouhani, NewFields Environmental Forensics; E. Littman, NewFields Environmental Forensics Practice, LLC

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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**

F. Ioppolo, M. Bellavita, J. Ross, S. Briemmer, ARCADIS

This methodology is based on targeted empirical 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 address contaminant regulations 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 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 thresholds target remediation limits.

**TH083**

**Fertilizers Environmental Exposure Tool: Advances in environmental exposure assessment of micronutrients in fertilizers**

S. Navía, Arche consulting / Laboratory of Aquatic Ecology, Evolution and Conservation; R. Paska, Yara Suomi Oy, M. Bjergøen, Yara International ASA; L. Delia Pietra, Fertilizers Europe; K. Oorts, ARCHE

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 (ECETO TRA, Chesar, EUSES) do not take into account interactions of the soil 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 is adapted to 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 other 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**

J. Chen, National Central University / Graduate Institute of Applied Geology; C. Liang, Fooyin University / Department of Nursing

Environmental 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 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 the Regulatory Context**

K. Szegedi, BASF SE, Agrarzentrum Limburgerhof / APF/EF; B. Jene, BASF SE / Environmental Fate

The implementation of novel results in environmental models which are used in regulatory context is a necessary step of scientific development. However, the between-contaminant 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 illustrated with a few examples. More general strengths, limitations and research needs will be discussed in the version control group. New model versions can be posted to the FOCUS DG SANTE (FORm for Co-ordination of pesticide fate models and their Use). One 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://eusolis.jrc.ec.europa.eu/projects/fooc-sdg-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 contrast to confirmed and scientifically accepted results, new ideas and hypotheses are not yet 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 large possible potential (bio)chemistry and behaviour, the vast number 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 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 (kdwwtp) and rivers (kdriver). 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 kdriver. 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 concentrations along the Llobregat river and at the influent and effluent of 2 WWTPs (R² = 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 deemed too small to be of environmental relevance. We found 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 also be 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 T. Gouin, Unilever / Safety and Environment Assurance Centre; J. Hermens, University of Utrecht / Institute for Risk Assessment Sciences; N. Kramer, Utrecht University / Institute for Risk Assessment Sciences

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 more 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 in 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 can 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 catonic 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 benzenalkonium 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 L. Henneberger and J. Corominas Catalan Institute for Water Research ICRA / Resources and Ecosystems; L. Bosch, Catalan Institute for Water Research ICRA / Resources and Ecosystems; J.A. Comas, L. Corominas, Catalan Institute for Water Research ICRA / Resources and Ecosystems

Relating in vitro to in vivo: bioavailability an essential element

TH090 Expanding the evaluation of the chemical activity hypothesis for toxicity assessment J.M. Armitage, University of Toronto - Scarborough / Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / Adjunct Professor, Department of Physical & Environmental Science/Department of Pharmacology and Toxicology; A. Orzaiztegi, ARC Arnot Research & Consulting / University of Toronto; T.N. Brown, TNB Research / Department of Chemistry; A. Celsius, Queens University; L.S. McCarty, L.S. McCarty Scientific Research & Consulting; D. Mackay, Trent University / Chemistry

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 Kow) ranging from -7.3 to 11.4. Scenarios of different degree of sampling can be obtained by changing the values of 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 K. Irwin, SETAC Europe / Aachen University / Institute for Environmental Research; Y. Jeong, KIST Europe

Environmental mixture toxicity results from exposure to undefined organic pollutant mixtures. The application of toxicity bioassays to measure this mixture toxicity 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 mixed samples 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 sample. To keep these applications viable, volatile chemicals have been tested in green test tube 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 toxicity. 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 polymer changes. To this end, 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 depleted mixtures causes 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.

T1H092 Exposure and Risk Assessment for Agricultural Applicator to Insecticide Chlorantraniliprole during Rice Cultivation in Paddy Field using Whole Body Dosimetry

J. Lee, Seoul National University / Department of Agricultural Biotechnology; Y. Shin, Seoul National University; J. Lee, Seoul National University / Agricultural biotechnology; M. Jung, E. Kim, J. Lee, D. Jeon, H. Ryu, X. Yuan, S. Baek, Seoul National University / Department of Agricultural Biotechnology; M. Rehan, S. Kim, Seoul National University / J. Kim, Seoul National University / Department of Agricultural Biotechnology. Chlorantraniliprole is a neonicotinoid 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/nack 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 (R²> 0.99) of calibration curve. Recovery (82–118%) of insecticide from various exposure matrices were reasonably 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 95% percentile of inner cloth exposure amount, 70 kg of body weight and 36 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.

T1H093 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 matrices, 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 generally 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 system 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 55% 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. GAČR 15-1651S2. Investigation of accumulation of persistent bioaccumulative toxic organic substances into aquatic organisms

Organic micropollutants in the environment: analytical challenges and engineering innovations (P2)

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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 mass spectrometric analysis 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. Replica 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 at >10 (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, diphenylhydradine, 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.

T1H095 Micro-pollutants along the river Rhine investigated by high resolution mass spectrometric approach

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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-targeted analysis allows detecting a much broader set of compounds (including unknowns). However, data processing remains still a challenging and time-consuming task, since typical 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 the 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, anti depressants and heart medications. Monitoring of other compounds, such as chlordane 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  
**JONIT NORDIC SCREENING OF EMERGING POLLUTANTS - EFFICIENT TOOL FOR DEVELOPING MONITORING AND REGULATIONS**  
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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, and emerging pollutants. For derivatization, 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 investigations or monitoring on national level. Screening is thus the first necessary step in the consensus 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 analytes 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 environment 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 national 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 method1 and workflow capable of detecting and characterizing novel PFASs in water by accurate mass and fragmentation flagging for perfluoro fragment ions (e.g. m/z 35, 33, 37, 77) identified independently. However, it was 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 liver from specimens collected in China (Tangxun Lake, Wuhan City and Yangtze River at Changdu) 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. CxFy, m/z range n>7). In polar bear serum, 4 new groups of PFASs (which were also found in fish livers) and 5 groups of new PCB metabolites were discovered. Our study demonstrates that this method may have significant applications, 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 use 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-CI-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-CI-OPEs were EHDPP, TBP and TBB. 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 180% below for TCPP to 4600% above for TBP. With the exception of TCPP, the model over-estimated water concentrations. Since the water concentrations were taken independently of the air concentrations, these results give 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 4099.1 kg and were significantly higher than emissions of PCBs and PBDEs calculated using the same model. We found that 70 and 60% of atmospheric loadings CI-OPEs and non-CI-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-CI-OPEs. Transfer from air to water was greater for these highly soluble compounds relative to PCBs and PBDEs. The difference between CI-OPEs vs non-CI-OPEs is consistent with their relative differences in water solubility. These model estimates are consistent with the hypothesis of long range transport CI-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-CI-OPEs were lost to degradative processes. Again, this difference is consistent with estimates of the persistence of Cl-OPEs in comparison to non-CI-OPEs.

TH099  
**Ecotoxicological risk assessment of traffic-related non-exhaust emissions**  

The demand for transport of both passengers and goods has been growing steadily and is projected to continue within Europe. This increased traffic load undoubtly have a major impact on the environment. In this study, we compared contributions to emissions of greenhouse gases 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 Daphnia Bioassay. Since these are very effective and cheap, the results may facilitate the 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 isomer (α-DDE) of 470 ng/L. The pre-concentration was 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 health risk of these compounds. 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 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 TANDER mass spectrometry T. Albanis, C. Kosma, University of Ioannina / Department of Chemistry; C. Nannou, University of Ioannina / Dept. of Chemistry; V. Boti, University of Ioannina / Department of Chemistry

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 WWTPs 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 main metabolites (N-Demethyl-Fluoxetine, O-DemethylVenlafaxine 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 appropriate commercial 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.


In the current study, a novel method optimization for estrogenic activity assessment of steroid hormones in water using DGT and the ERE-CALUX bioassay was developed to determine the estrogenic activity of steroid hormones in water. The DGT-CALUX method was developed for the simultaneous determination of estrogenic activity of steroid hormones in water. The method was validated for the determination of estrogenic activity of steroid hormones in water and the results showed that the method was accurate and precise with excellent linearity and detection limits for the tested concentration.
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, VM7Luc-HE2 cells (formerly BG1Luc-HE2) as ER-EALUX biosay 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 ER-EALUX cells. XAD was shown to be sufficient in efficacy for the tested substances and the studied soil. The bioassay measured effective diffusion coefficients of E2 in agarose gel diffusive gel was 4.7 × 10^{-10} cm²/s at 25 °C, which is slightly lower than the theoretical one (5.2 × 10^{-10} cm²/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 (0.14-0.10 ng/L). Independent measurements (0.001-0.1M) and dissolved organic matter concentrations (0-30 mg/L). This study demonstrates that DGT combined with ER-EALUX is an effective tool for pre-concentrating steroid estrogens and possibly also in the monitoring of estrogenic 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 supplier specific data formats be handled? Would governmental initiatives compete with already ongoing research driven processes? Norwegian authorities invite 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 STP which the entire city's waste water comes in and compare each other. In this study, which is one of the first studies on the presence and daily variation 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, benzoylgesoline, codeine, MDA, MDEA, MDMA, amphetamine, methamphetamine, morphine, THC, THC-OH, THCCOOH) has been developed and used for the analysis. Codein, amphetamine, methamphetamine and Benzoylgesoline were detected in both sampling stations. While cocaine was detected in 7 samples in Eastern STP, it was not found 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 polychlorinated biphenyls (PCBs) and 14 dioxin-like PCBs, namely: α-HCH, β-HCH, γ-HCH, Δ-HCH, Hexachlorobenzene (HCB), Heptachlor, Aldrin, Dieldrin, Dieldrinhexoperoxyl-A, Heptachloroperoxide-B, pp'-DDE, pp'-DDE, pp'-DDE, op'-DDE, op'-DDE, op'-DDE, Endosulfan-1, Endosulfan-2, Endrin, Mirex, PCB-77, PCB-81, PCB-105, PCB-114, PCB-118, PCB-123, PCB-126, PCB-135, PCB-149, 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 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. The results of the studied soil sample indicated the presence of 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 like PCBs 126, 157, 169, 170, 180, 189 were not detected in any of samples. Aspectives, 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 88.8% 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 Nubarakshen 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.

TH110 Occurrence and spatial distribution of six endocrine disrupting compounds in surface- and groundwater 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 (estrene - E1, β-estradiol - E2, 17α-ethinylestradial - EE2, bisphenol A - BPA, perfluorooctanoic acid - PFOA, perfluorooctane sulfonate - PFOS) was assessed in the Apenninic rivers and groundwaters of the Romagna area (North 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 groundwater, whereas in surface waters all classes of compounds were detected at concentrations above the method limit of quantification (MLQ). 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 MLQ in all samples, whereas E1 and EE2 reached concentrations up to 39.74 ng/l (E2) and 28.01 ng/l (EE2) in surface waters. The highest estrogen values (≥ 20.54 ng/l, median value) were located in those areas where illicit drug use is prevalent, 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 potentially hazardous to human health and the environment. The purpose of this research was to investigate the occurrence of benzamides, 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 phase was composed of 50:50 water-acetic acid (eluent A) and acetonitrile (eluent B). The analytical method was applied to water
samples from the Moci Guaca River in the State of Sao Paulo and Itapirucu 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) F. Cevik, University of Cukurova / Department of Fisheries; E. Guzel, University of Cukurova; N.G. Dagioglou, University of Cukurova / Department of Forensic Medicine

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. Pharmaceutical compounds are toxic and carcinogenic, and have the potential to disturb human health should be under consideration and further studies should be done.


The Ruhr area in North Rhine-Westphalia, the biggest contamination 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(I)/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 vary (up to 10 times). 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 slightly 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 E. Guzel, University of Cukurova; N.G. Dagioglou, N. Gokce Daglioglu, University of Cukurova / Department of Forensic Medicine

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: wastewater, stormwater discharges, interacting with various pollutants, and leaching from landfills. 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 period of April - November 2014. A novel full-scan SPE-UHPLC-Orbitrap-HRMS method was used and the analysis of 95 compounds from various of drug groups including pain killers, antibiotics and antibacterial, cardiovascular drugs, lipophilicidemis, central nervous system drugs and hormones androgenic and anabolic steroids was carried out. Some compounds were detected in Ivriz, Ayranci and Godet Dam Lakes, respectively. Lidacon 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 Have plasticizers and additives penetrated our aquatic environment? S. Huysman, Ghent University; F. Voronckyghem, Ghent University (UGent) / Sustainable Organic Chemistry and Technology; H. Van Langenhove, K. Demestere, Ghent University / Sustainable Organic Chemistry and Technology - Research Group EnVOC; L. Vanhaecke, Ghent University / Veterinary Public Health and Food Safety - Laboratory of Chemical Analysis

Phthalates and phenols are a large group of ubiquitous, high-volume industrial compounds, besides the list of aforementioned target compounds. Finally, this work presents a novel full-scan SPE-UHPLC-Orbitrap-HRMS method was 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 Uluborl Dam Lakes; 9 and 15 kinds of human pharmaceuticals and personal care products were identified in these 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 due to a 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.
given a.o. the challenges with respect to sampling and ultra-trace (<50 pg 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 whole-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 off shore of Zeebrugge. 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-Exactide 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 mostly of the compounds. The concentrations were quantified from analyte-rich matrices 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 NewSTEPS project (BR/143/2/AU/NewSTEPS). 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-Exactide and the automated SPE equipment, respectively.

TH116 Occurrence of oxygenated poly cyclic aromatic hydrocarbons in mussels at the Belgian coastal zone
B. De Witte, ILVO / Aquatic environment and quality; C. Walgraewe, Ghent University / Department of Sustainable Organic Chemistry and Technology; K. Demeestere, H. Van Langenhove, Ghent University / Sustainable Organic Chemistry and Technology - Research Group EnVOC.
Oxygenated polycyclic aromatic hydrocarbons (oxyPAHs) are polycyclic aromatic hydrocarbons (PAHs) containing carbonyl 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-MS² based analytical method is developed, validated and applied to determine 6 oxyPAHs (naphthalene-1-ol, 9H-fluorenone, anthracene-9,10-dione, 7H-benzo[de]anthracene-7-one, naphthalene-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 in the easternmost mussel samples 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.¹

TH117 Organophosphate flame retardants in L’Albufera Natural Park (Valencia, Spain).
M. Lorenzo, Universitat de València; J. Campo, University of Valencia; Y. Pico, University of Valencia / Medicine Preventive;
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 that they can reach in the 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 surface waters and sediment. The eleven PFRs selected were tri-n-propylphosphate (TPhP), tri(2-chloroethyl)phosphate (TCEP), tris(2-ethylhexyl)phosphate (TEHP), tris(2,3-dibromopropyl)phosphate (TDBPP), tri(n-undecyl)diphenylphosphonate (TUDP), tricyclohexylphosphate (TCP), tris(2-dichloroethyl)phosphate (TDCIPP), tris(2-chloroethyl)phosphate (TCEP), tris(2-chloroisopropyl)phosphate (TCIPP), triphenylphosphate (TPP), tris(2-butoxyethyl)phosphate (TBP) and tributyl phosphate (TBP).

The extraction of 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, TCIPP and TCEP were the compounds found at higher concentration. In soil and sediment samples high concentrations were found for TCIPP, TDCIPP and TDBPP. Finally, in biota samples, TCIPP was found at the highest concentration. Relationships between high PFR 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 Chlorobenzenes in organs of fishes from Lake Dongting
G. Zhao, China IHWIR
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 wet weight to 7811.2 (catfish) ng/g wet weight, 1.3, dichlorobenzen, 1.2-dichlorobenzen 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 2432.3 (cruccan carp) to 3329.6 (catfish), 2557.9 (catfish) to 4640.0 (yellow catfish) and 16281.1 (yellow catfish) to 4667.8 (cruccan carp) ng/g wet weight, respectively. CBs from the production and use of Sodium pentachlorophenol 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
S.B. Gutwurtz, University of Windsor / Department of Geography; N. Gandhi, University of Toronto / Dept of Earth Sciences; K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; T. Kolic, Ontario Ministry of Environment and Climate Change; K. MacPherson, E.J. Reiner, Ontario Ministry of Environment and Climate Change / Laboratory Services Branch; S. Bhavsar, University of Toronto
Polychlorinated naphthalenes (PCNs) were one of the first groups of synthesized chemicals, and their high-volumne 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 wet weight contributing 0.2-54% to TPCH. PCN-52/60 contributed most (34%) followed by PCN-53/64/68 (22%) and PCN-66/67 (17%), however PCN-52/60 contributed 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+dPCBs) ranged (25th-75th percentile) from 4.7-12% to 4.7-12%, however only 9 congeners contributing to 2,3,7,8-TCDD Toxic Equivalent (TEQ) concentration. 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
B. Park, National Institute of Agricultural Sciences, RDA / Chemical Safety; S. Lim, S. Ryu, National Institute of Agricultural Sciences, RDA; G. Choi, NAAS RDA
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 residues 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
Y. Yamamura, Chiba University of Technology / Department of Creative Engineering. Neonicotinoid insecticides are widely used in Japan. Seven neonicotinoid insecticides such as Imidacloprid, Acetamiprid, Thiacloprid, Clothianidin, Dinofeturan, Thiamethoxam and Nitenpyram are popular. Their usage began from the beginning of 1990 and is increasing till 2009 in Japan. Recent annual total usage of these 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
M. Saint-Hilaire, Université de Lorraine UL / URAPFA INRA; A. Fournier, Université de Lorraine UL; G. Rychen, C. Feidt, Université de Lorraine UL / URAPFA INRA; C. Ithavong, J. Parinet, ANSES / Unité PBM
Chlordecone (or kepone) is an organochlorine pesticide intensively used against leishmaniasis in dogs and against malaria in humans. Chlordecone’s metabolites in bovine, caprine and caprines’ metabolites in bovine, caprine and caprine liver tissues were not detected. These results showed that the residue in paddy and upland soils were lower level than bioaccumulation occurring.

poor recoveries were found. A more specific method has been developed to determine the concentration of chlordecone in livestock liver: it involves a better extraction solvent for chlordecone 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 chlordecone. As for the conjugated metabolites of an enzyme called hepatic reductase, it was used to detect the concentration 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
J. Kim, Y. Oh, S. Choi, Korea Institute of Korea KIT; S. Hong, National Academy of Agriculture Science, Rural Development Administration; J. Seo, Korea Institute of Korea KIT
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 spray dryer. 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 to be sent to check for the homogeneity of the cherry tomato analytical reference material was confirmed that it was in good agreement. The uncertainty(±u) 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 slob(b)1 values were smaller than the corresponding values of [t0.05,n,0.05(b)1] value. 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, tebuconazole, tetraconazole, pyraclostrobin
J. Kim, Y. Oh, S. Choi, Korea Institute of Korea KIT; S. Hong, National Academy of Agricultural Science, Rural Development Administration / Chemical Safety; J. Seo, Korea Institute of Korea KIT
A cherry tomato analytical reference material for pesticide multi-residue analysis(buprofezin, tebuconazole, tebuconazole, 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 spray dryer. 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 to be sent to check for the homogeneity of the cherry tomato analytical reference material. The uncertainty(±u) 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. For all target pesticides, the slob(b)1 values were smaller than the corresponding values of [t0.05,n,0.05(b)1] value. 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.

Fiber optic color sensor for determination of chemical parameters in different matrices
A. Rösch, Eawag / Environmental Chemistry; M. Gottardi, N. Cedergreen, TH128
A STRATEGY TO MITIGATE IN A SUSTAINABLE WAY AMMONIA EMISSIONS FROM UREA COATING. A LOW-TECH APPROACH TO IMPROVE UREA STABILITY IN THE FIELD
S. Da Rosa, University of Pampa University of Pampa, Foundation for Agricultural Development FADU)
A strategy to mitigate ammonia emissions from urea coating. A low-tech approach to improve urea stability in the field.

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. The sensor would ensure safety and efficiency, a property that is especially important in the field where high concentrations of nitrogen are handled. It is expected that the use of such a sensor in the field will significantly reduce ammonia emissions.
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/MS/MS

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The purpose of this study is to investigate pesticides and other toxictants 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. 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 performed for the identification of suspicious toxictants 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 toxictants in grain tea. Finally, target analysis followed for the confirmation with the standard materials for tentatively identified toxictants via suspect/nontarget screening. As a result from suspect screening, four fungicides were identified by isoype pattern and fragment analysis with help of MS/MS DB (e.g., MassBank, M2Cloud) 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 toxictans 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 with phototherapy 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, nitrate, sulfate, cation Ca**2+** 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 developed method. The instrument used for 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. This 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 stimulates 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 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 11 of 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-fungality 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 self-selected substrate (the stilbene oxide 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 (cyprenoxalone, epoxiconazole, fluconazole, ketocanazole, 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 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 led 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),
TH132 Pesticides in paddy soil and rice from the Kingdom of Thailand: Fate and risk assessment

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Abstract: The major source of income of rice is the paddy fields in northern (Khamphangphet 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 m² 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 and the paddy rice extract were loaded into an alumina cartridge and a florisor 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 0.51 to 109.10 ng g⁻¹ dw (mean: 25.12 ng g⁻¹ dw). Considering DDT and its metabolites; o,p'-DDT, p,p'-DDD, and p,p'-DDE 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 was similar in the paddy field analyzing by factor analysis. It can be concluded that extracting mirex, o,p'-DDT, p,p'-DDD, and p,p'-DDE was mixed in 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 a pesticide law and a list of non-target organisms and a system of risk assessment (ERA) scheme under realist treatment regimes. In a 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 sprayeries 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 sprayeries 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 designated as representative for 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 scenarios into typical treatment regimes as well as effect estimations from appropriate mixture toxicity models in the next steps.

TH314 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 a single fish species. During the Design of Toxicity (DTDs); 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 account for toxicological effects 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 fish of food 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.

TH315 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 (a Microtox® test system). Experimental results showed a relevant toxicity for almost all of the single QACs, with IC₅₀ values lower than 1 mg L⁻¹. The comparison between experimental and predicted IC₅₀ 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 both binary and multimixture components 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 multimixture compounds (7 out of 11 tested mixtures) a good agreement between experimental and predicted IC₅₀ was observed, as confirmed from the application of the Model Deviation Ratio (MDR). In some cases, from deviations from the expected behavior, it is possible to identify key components (potential interactions) at concentrations in the order of hundreds of 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.

TH316 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 representatives of emerging contaminants to study the synergistic toxicity effect with the fluorouraciluracil perfluorooctanoate sulfonate (PFOS) in two biological models, Danio rerio and the Oryzias latipes (medaka). 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 eluetheroembryo 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 synergistic effect on different biomarkers of exposure and toxicity. Moreover, the tested combination of PFOS (6 µg/ml) and carbamazepine (25–100 µg/ml) was determined as toxic to both species in early life stages.

TH317 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. Recently, 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 databases respectively available models (O. concinna, O. latipes) with our findings. Firstly, 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. This is further supported by our analysis of 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.

TH318 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 resources - UFZ / Effect Directed Analysis; R. Altenburger, UFZ 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 monitoring assesses individual chemicals, to 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 waters (Meier et al. 2014). Steele and measure 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 environmental contaminants from multiple sources (EBT). In this work we therefore undertake to (i) identify 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* J. Rana, D. Patel, Jai Research Foundation / Ecotoxicology; N. Khan, Jai Research Foundation / Chemistry; N. Reddy, Veer Narmad South Gujarat University, Surat / Department of Biosciences 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 near 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 daphnids was 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 EC50 values were 24.58 and 0.04% v/v, respectively. The clinical symptoms and EC50 value indicates that the effluents have very toxic effect on *Daphnia magna*. 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 R. Morgado, University of Aveiro / Department of Biology and CESAM; M.D. Pavlaki, University of Aveiro / Department of Biology; A.M. Soares, Universidade de Aveiro / Department of Biology & CESAM, 3810-193 Aveiro; S. Loureiro, Universidade de Aveiro / Biology Groundwater constitutes 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 qualifying 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 ecosystem health. Identifying the composition of emerging pollutants in 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 tiered 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 Combined Concentration Addition (CCA) (Wolter et al. 2013) to adaptively simulate the interaction between contaminants. The consequences groundwater management are discussed.

**TH141**
Devising spatial composite indicators to identify environmental health risk areas in Walloon region S. Habran, ISSeP / Environment and Health; E. César, ISSeP / Dept Environmental Health; M. Veschvens, Inst. Scientifique de Service Public; S. Remy, ISSeP / Dept Environmental Health Developing an environmental 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 is used to identify, on a regional scale, geographic areas where hotspot exposures exist and determine the 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 pollutants and stressors.

**TH142**
Adaptation of the WHO/IPCS framework on mixtures assessment to regulatory environmental risk assessment practices K. Yamazaki, Ministry of the Environment / Environmental Health 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 2015 with a tiered approach should be useful considering the mixture 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 adopt 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 M. Zeman, ARCHE; S. Hubbard, SAHC0 Ltd; E. Van der, Architecture; K. Joly, C. Vleminckx, Institut Scientifique de Santé Publique / Bruxelles; B. Lacasse, European Copper Institute Inorganic UVCBs (iUVCBs, Substance of Unknown or Variable composition, USV) are poorly characterised complex mixtures and articles (i.e., final products). This is due to the fact that mixture health risks are properly controlled by calculating the risk characterization ratio (RCR), and that regulatory perspective (RPs) are also calculated through the web; and to check which components are critical to the mixture toxicity in order to risk management. This poster presents a pragmatic way forward to combined risk assessment factors and efficient solutions in terms of risk management. This is achieved 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 Combined Concentration Addition (CCA) (Wolter et al. 2013) to adaptively simulate the interaction between contaminants. The consequences groundwater management are discussed.
conservative tier is based on the summation of the associated RCRRs (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 characterisation under REACH based on ECHA Guidance for assessing the risk of biocidal products. Subsequent tiers consider target organ toxicity and mechanistic data and adds RCRRs 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

H. Jeong, J. Kim, KIST Europe / Environmental Safety Group

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, risk is properly identified 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 study on 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 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 effect of environmental mixtures; however, they are not in accordance with regard to the predictability of mixture toxicity for chronic effects and effects at higher levels of biologic 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 dataset of PPP in tank mixtures is analyzed to determine concentration related endpoints 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 predictiveness of existing risk assessment schemes based on single products and will develop 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 would be extremely time-consuming due to the extremely large number of combinational 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 used 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.
ingestion 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, OCIs 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 the knowledge and inherent uncertainty associated with specific chemical categories and all available ingredients in cleaning products. For each CTD, threshold levels (95% CIs) were identified at 1st, 5th, 10th, 50th, 90th, 95th, and 99th 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 (reproductive 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 Metal exposure concentrations in the test media were measured. Concurrently, the carbon dioxide levels, particle size distribution, air relative humidity, and temperature were monitored. Chemicals commonly used as cathode materials for printed batteries (electrochemical inkjet printing) such as lithium-ion batteries (LIB) of which Li-phosphate (LFP) and lithium nickel manganese cobalt oxide (NMC) are the most common. 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. Where default UFs are sufficient for various chemical uses or specific chemical classes remains understudied, particularly for ingredients in cleaning products. Therefore, we systematically examined publicly 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 Initiative database. We employed a probabilistic chemical toxicity distribution (CTD) approach to identify likelihoods of exceeding the thresholds, and adjusting the uncertainty factors over all dose descriptors for specific chemical categories and all available ingredients in cleaning products. For each CTD, threshold levels (95% CIs) were identified at 1st, 5th, 10th, 50th, 90th, 95th, and 99th 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 (reproductive 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 key trade association that represents the cleaning product manufacturers. The inclusion of approximately 90% of all the major 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 each 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, ICCFR, 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, oral, dermal, and inhalation studies as opposed to LD50 data from acute studies; and data with a Klimish reliability rating of at least 1 or 2. Where data where 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 calculation of the results of 53 articles published between 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 Additive (IA) models, 12 other alternative models were tested. 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 (ecotox)testing of multi-component metal oxide nanomaterials

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Lead-exposed workers may suffer adverse health effects under regulated blood lead levels. In the context of potential health risks, a critical priority is the development of 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 41%. The interpretation of the model was feasible for the given data and model assumptions. The model predictions as compared to Pb biomonitoring data has been reported in previous work. Also, the accuracy of the prediction was evaluated using the cross-validation method to compare the individual pharmaceutical and the mixture effects. We demonstrated that the model can be considered suitable for high-risk scenarios that account for subject mobility, i.e., duration at the different considered locations. The assessment of the mixture experiments and interpreting the outcomes.

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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
T. Fioren, VITO; P. Ciffroy, EDF / LNHE Department; M. Van Holderbeke, VITO NV; A. Staendaert, VITO; C. Cornelis, VITO / Environmental Risk and Health; C. Brochot, INERIS / Models for Toxicology and Toxicology METO; E. Johansson, Facilia AB; J. Bierkens, VITO

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 to 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 (Fioren 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-exposed workers: a risk assessment in Taiwan
Y. Lin, National Taiwan University / Department of Bioenvironmental Systems Engineering; N. Hsieh, Texas A&M University / Department of Veterinary Integrative Biosciences; C. Liao, National Taiwan University / Department of Bioenvironmental Systems Engineering

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 standards. Our results indicated that HI for total lead-exposed workers was 0.50, 0.31 and 0.16 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 \(\text{dL}^{-1}\). We conclude that long-term exposure to lead in the workplace was significantly associated 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
K. Báňiová, Masaryk University; A. Sharma, M. Chropořevá, Masaryk University / Research centre for toxic compounds in the environment; P. Přibylova, Masaryk University / RECETOX Research Centre for Toxic Compounds in the Environment; R. Prokes, Masaryk University / Research centre for toxic compounds in the environment; O. Audy, J. Kohoutek, Masaryk University / RECETOX, P. Cupr, Masaryk University / Research centre for toxic compounds in the environment

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD) are flame retardant compounds widely used in the furniture and electronics industry. The aim of this study is to assess indoor PBDE and HBCD concentrations and risks associated with them 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 time. The main pathways for the detection of concentrations and toxicity are exposure assessment and risk assessment of PBDE 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 simultaneously: passive sampler registering the polycarbonate 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 for biotransformation, toxicity effects, and persistence, their potential 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), dibenzyl phthalate (DnBP), 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 contributions of DnBP were 29, 26, and 74% in house, workplace, and daycare center, respectively. DnBP 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), dibutyl phthalate (DnBP), DnBP, and DEHP. Median contributions (%) to the TDI were 0, 1, 43, and 25% for DEP, DnBP, DnBP, and DEHP, respectively. Considering no correlation between levels of dust and urine, and low representation of dusts containing the polycarbonate foam sorbent 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 DnBP.

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.) [16]. To determine the toxicity of pollutants, the 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-disruptor activity (oestrogenic 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 and cytotoxicity 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 endocrine, 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 biosoays 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 H. Wong, University of York / Environment; C. Brown, University of York / Environment Department; C. Ramwell, D. Garthwaite, Fera Science Ltd 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 downhill (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 system 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 K. De Brouwere, VITO NV / Health; J. Bierkens, VITO; G. Koppen, VITO / Environmental Health and Risk; T. Van der Donckt, University of Ghent / Toxicology and Public Health

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 EU REACH pre-registration phase (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, the main goal of the project, 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 project, 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 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 for orchard systems and outdoor workers in agricultural and horticultural sector. The dual 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.

TH160
Accumulative effects of polycyclic aromatic hydrocarbons (PAHs) in the environment on telomere length N. Lin, Peking University / Environmental Geography; Y. Ren, B. Wang, S. Su, Z. Li, Peking University; T. Shu, Peking University / Laboratory for Earth Surface Processes College of Urban and Environmental Sciences

Exposure to polycyclic aromatic hydrocarbons (PAHs) is believed to be associated with increased risk of adverse health conditions. During the exposure period, cell division is promoted for tissue renewal and function maintenance, which is reflected in the shortening of telomere length.

TH161
Biomonitoring of human exposure to benzene and toluene in oil extraction area of Muanda, Congo M. Creta, KU Leuven / Environment and Health; R. Duca, KU Leuven / Center of Environment and Health; H. Vtiens, KU Leuven / Environment and Health; M. Onken, J. Vanoorbeek, W. Reniers, J. Vanoortbeek, B. Nemery, KU Leuven / Environment and Health

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 intact 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 dicumyl acid (as biomarker for toluene exposure), 1,3-muconic acid (as biomarker for benzene exposure) were determined. For dicumyl 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 “oils wells” group; 240 mg/L (range: 50 - 1249 mg/L) for “oil handling” group. The concentration of t-MTA in urine was 0.05 mg/L (range: 0.05 - 0.15 mg/L) in Mangrove group; 0.15 mg/L (range: 0.12 mg/L) in “oils wells” group; 0.4 mg/L (range: 0.27 mg/L) in “oil handling” group. The concentration of SPMA in urine was 0.24 mg/L (range: 0.05 - 0.4 mg/L) in Mangrove group; 0.19 mg/L (range: 0.05 to 6.7 mg/L) in “oils wells” group; 0.51 mg/L (range: 0.11 - 6.85 mg/L) in “oil handling” group. In conclusion, urinary biomonitoring had revealed that the ‘oils wells’ group and the ‘oil handling’ group have shown urinary concentrations slightly higher than the ‘mangrove’ group. Nevertheless, the study has shown a high degree of increased risks for human health in relation to oil extraction activities in Congo.
A probabilistic risk assessment for the impact of airborne paracetamol-associated exposures on Parkinson’s disease

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BACKGROUND: Evidence showed that the herbicide paracetamol (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 derived based 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 (39.8–63.1), respectively. In Taiwan, the largest PQ exposure concentrations occurred in its positive trend during 2004–2011, with the PQ contributing nearly 21% and 24%, respectively. PD prevalence risks of PQ were identified 70–79 yr and ≥ 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. 

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 National 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 (WRP) 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 for the purpose of toxicity and exposure and on the sales and on the uses of the molecules in Belgium and in the Wallon 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 Quebec 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 presence 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 was expressed as a percentage of the sales and usage and as a percentage of the sales and on the uses of the molecules in Belgium and in the Wallon Region. The calculated PRIs are used for monitoring the environmental risk to human health of active substances in Wallonia. In Wallonia, the calculation of toxicological risk indicators needs the knowledge of the sales and sales of PPP. PRIs might allow the regional governments assessing the effectiveness of the measures taken within the WRP for the reduction of risks associated with the use of PPP for public health.

Assessing the food safety risk in children exposed to organophosphate pesticides in Taiwan

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Baby and adult populations have large differences in eating habits. Baby food cooking method is 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 National Health and Nutrition Examination Survey (NHANES) using the U.S. 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 >4AD 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, thereby warning people for food items to 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 6.2% ADI, which was acceptable risk.

Assessing BPA intake from canned food consumption based on web survey

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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 is 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 human 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.

Toxicological properties of PM10: relationships with markers for wood burning and traffic and association with health biomarkers in an adolescent population of Flanders

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The study commissioned by VMM within the framework of JOAQUIN investigated the toxicity of particulate matter (PM10) 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 PM2.5 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 PM10, mutagenic and oxidative potential of PM10; (2) to verify spatial differences in the responses; (3) to identify pollutants which are associated with the observed biological effects. RESULTS: Correlation of DCX and caspase 3 with PM2.5 concentrations and inhibition of dopaminergic cell viability was constructed by the Hill equation model. 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. 

Oxidative potential of PM10 collected in the urban area was seen. Compared to the background location a significant increase in inflammatory mutagenic and oxidative potential of PM10 collected in the urban area was seen. Compared to the background location a significant increase in mutagenic and oxidative potential of PM10 collected in the urban area 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 PM10 played an important role in cytotoxicity, pro-inflammatory cytokine induction, oxidative potential and oxidative DNA damage. Markers for wood burning and traffic were associated with increased mutagenic and oxidative responses. BC and EC correlated with cytotoxicity and with the
Improving the environmental assessment of complex composition substances and mixtures for Chemicals Management (P)

TH169

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 substances manufactured or imported in quantities of >10 tonnes per year. Meeting 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 depots, 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). Compiling with regulation while remaining competitive in the European market and the circular economy objectives represents a big challenge for the metals industry.

TH168

CRANCS2: promotion of a new methodology to deals with Natural Complex Substances type 2
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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 terpenic 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 NCS2, 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 classification or structural formulas, reaction steps, physicochemical properties, or spectral data. A methodology allowing structural description of UVCB substances has been developed. Defining an inambiguous structural description aims to complete the following two tasks: to allow performing of in silico hazard assessment, where structural information is essential, and to contribute towards fulfilling the requirements of implementation of any chemical relation legislation. It is also envisioned 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 an added toxic effect attributed to the UVCB as a whole. The methodology, based on the generic substance identifiers, allows coding, generation, and query 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 applied to Ames mutagenicity assessment of a petroleum UVCB substance.

TH171

Practical Considerations for PBT Assessment of Complex Mixtures with Difficult Properties
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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 proposed approach to address 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 bioconcentration 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 approaches 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 ?
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Under the REACH registration program several kinds of substances are considered: mononconstituents, 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. In Fragrance chemicals can be of natural origin or synthetic and one particular group of natural fragrances that falls under the title of
multiconstituent/UVCBs, 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 required data for registration of these substances, several testing strategies have been developed based on available guidance documents (CLP, R7a,b,c, R11, EFEFO/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.

TH175 Evaluating uncertainties in the risk assessment framework for petroleum hydrocarbons
A.D. Redman, Exxon Mobil BioMedical Sciences / Toxicology and Environment Science Division; M. Leon Paumen, ExxonMobil BioMedical Sciences / ExxonMobil BioMedical Sciences TES Division; K. den Haan, CONCAWE / Petroleum Products & Safety; E. Vaiopoulou, CONCAWE Risks (RA) evaluation, use relative to conservative chronic effect-based hazard thresholds. Variability in the exposure and hazard sides of the RA framework are introduced in order to the RA framework to appropriately 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 relative to hazards 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 useful basis for deriving a 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.

TH174 Determination of PNEC values for risk assessment of natural complex mixtures: options and limits
C. Durou, R. Samsara, P. Thomas, CEHTRA SAS Under the REACH registration program several kinds of substances are considered: monocomponents, monomeric substances & UVCBs. Across these substances (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 the fragrance that falls under the title of multicomponent UVAUVCBs, 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 for the ERA calculations. Regarding to 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 the WAF approach is not associated with the principles of the EU REACH rules for risk assessment and a WAF approach 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 database 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.

TH173 Assessing the Bioconcentration Potential of Fragrance Ingredients in Fish via Dietary Exposure with Internal Chemical Benchmarking
C.-J. Chen, Stockholm University / Department of Environmental Science and Analytical Chemistry; K. Löfström, Stockholm University / Department of applied environmental science; M. Adolfsen-Erici, Stockholm University; M. MacLeod, ITM - Stockholm University / Department of Environmental Science and Analytical Chemistry; M. McLachlan, Stockholm University / Environmental Science and Analytical Chemistry (ACES) 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 up-take experiment as the test substance was evaluated, 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 days’ depuration. Previous studies documented that the benchmarking technique can reduce the length of exposition of the experimental organism. The aim of this study was evaluating relative validating both theoretically and on a real case study and then applyed to Ames test and benchmarking chemical compounds (including α-pinene, camphene, limonene, 2,4-bis(2-hydroxyethyl) acetate (Verodx), cashmeran (DPMI), cyclohexyl salicylate, acety lcedrene, musk xylene, globulone and decamethyleneoctanosiloxane - D5, hexylchlorobenzene - HC8 and 2,2',5,5’-tetrachlorobiphenyl - PCB52) via single contaminated dietary exposure and with benchmarking technique. Results show that the concentration of chemicals in the fish followed the first order kinetic model with k ranging from 0.0220 d^-1 (musk xylene) to 0.5999 d^-1 (Verodx). The BCF values derived from the calculated uptake rate constant (k) using the Arnot & Gobas model divided by the k0 for the tested chemical and were in the range of 230 (Verodx) – 6300 (musk xylene) L kg^-1. The benmarkd BCF (BCFmax) ranged from 240 L kg^-1 (Verodx) to 7500 L kg^-1 (musk xylene). These BCF data agreed well with the previous 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.
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 difficulties since many of these substances are 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. This has been established and internationally recognised in guidance documents as an accepted methodology for testing these products (OECD, IMO, ICHA, 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 physicochemical 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. CONCAWE (1993). Report 92/56: ecotoxicological testing of petroleum products.

TH179 Understanding WAF results used for the assessment of aquatic toxicity (CRANCS project) P. Bichroel, KREATs; P. Thomas, CEHTRA SAS Novelty: 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 rate 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, KREAT’s has developed an iSafeRat® calculation method which can accurately predict the aquatic toxicity to fish, daphnids 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 studied in order to test the applicability of the WAF in risk assessment of complex chemicals. 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 R. Smith, wca; G. Whale, Shell Health / Shell Health Risk Science Team; P. Weissbrod, wca; A. Eldon 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 thickener substance when in a surfactant solution. The European Thickeners Consortium (ERTGC) 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 the thickener substance when it is in situ in base oil. The additional tests which the ERTGC 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 ERTGC found that a ‘pass’ for substrates 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 ERTGC have proposed an ‘alternative approach’. Where the weight of evidence to support the use of the isolated substance is compelling, 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 ERTGC Technical Working Group for their assistance in developing the alternative approach.

TH181 Physicochemical and biological characteristics of leachate from biochar produced from malt spent roots M. Dormoussoglou, University of Patras / Department of Biology University of Patras; S. Daulianis, University of Patras / Biology; A. Tsooulafta, University of Patras; I. Konstantinou, University of Ioannina / Chemistry; D. Vlastos, University of Patras / Department of Environmental and Natural resources Management; M. Antonopoulou, A. Lavranou, University of Patras; H.K. Karapangioti, University of Patras / Chemistry Department; I. Manariotis, University of Patras / Civil Engineering The present study investigates the physicochemical composition of water aliquots derived from biochars produced from the pyrolysis of malt spent roots, in comparison with the composition of water aliquots from soil samples. The concentration of water aliquots was performed using the Water Accommodated Fraction (WAF) methodology for testing these products (OECD, IMO, ICHA, 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 physicochemical 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. CONCAWE (1993). Report 92/56: ecotoxicological testing of petroleum products.
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 (Lambertia 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 organisms may be affected. 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 such interactions. It is not clear why 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 EC50 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 affect the effects described for the smaller sizes. 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 this plastic found in sludge, approximately 80% was a type of polyethylene. Other studies have shown that 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 bioaccessibility of microplastics in 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/filter 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.

TH185
Sludge as an origin of microplastic in agricultural farmlands
N.V. Alst, M. Simon, Aalborg University, D.A. Stephansen, J. Vollertsen, Aalborg University / Civil Engineering
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 in wastewater sludge 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/filter 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.

TH186
Microplastics in a UK canal system: Abundance, characterisation and impact on freshwater microbial communities
R.M. Adams, The University of Birmingham / Geography, Earth and Environmental Sciences; I. Lynch, University of Birmingham / Geography Earth Environmental Science; J. Saüer, The University of Birmingham / Geography Earth Environmental Science
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). In there is thus an urgent need to address this concerning knowledge gap by assessing the occurrence of MPs, fate and impacts of both MPs and nanoplastics (NP) 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® multimeasure 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 synergetic 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
A. Bellingeri, E. Bergamini, University of Siena / Department of Physical, Earth and Environmental Sciences; P. Redondo Hasselener, Wageningen University / Aquatic Ecology and Water Quality Management; W. Beekman-Lukassen, F. Gillissen, Wageningen University Research / Aquatic Ecology and Water Quality Management Group; I. Corsi, University of Siena / Department of Physical Earth and Environmental Sciences; A. Koelmans, Wageningen University / Aquatic Ecology and Water Quality Management
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 unique properties which give them specific toxic potential depending on surface characteristics and interactions with the surrounding medium. Carboxylated poly styrene nanoparticles (PS-COOH NPs) of a nominal size of 70 nm have been tested alone and in combination with copper nitrate to freshwater microalgae 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, a NP-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 Lenna minor root growth and root cell viability
G. Kaklitskova, A. Žgajnari Gotvajn, University of Ljubljana / Faculty of Chemistry and Chemical Technology; A. Kladnik, University of Ljubljana / Biotechnological Faculty; A. Jemec, University of Ljubljana, Biotechnical Fac. / Department of Biological Sciences; A. Zgajnari-Gotvajn, University of Ljubljana / Faculty of Chemistry and Chemical Technology; T. M. Zpol, University of Ljubljana / Institute of Chemical Engineering; J. P. Kaiser, University of Ljubljana / Department of Biology.

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 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
C. Scherer, L. Rongstock, M. Dann, Goethe University Frankfurt am Main; M. Wagner, Goethe University Frankfurt / Laboratoire Interdisciplinaire des Environnements Continentaux, CNRS UMR 7360; S. Lambeth, Goethe University Frankfurt am Main.

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), sometimes with the surface of the samples remaining 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 were emerged: environmental viruses or contaminated MP, 100 MP (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 150 µg TBT/L of 0.15 µg TBT/L; 10, 50 and 100 particles L⁻¹. 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 such as the fate and fragmentation profiles of MPs in a freshwater model system.

TH190 Freshwater microplastics and competitive co-occurring pollutants
K. Reilly, The University of Birmingham / Environmental Health; J. Sadler, The University of Birmingham / Department of Geography and Environmental Science; I. Lynch, University of Birmingham / Geography Earth; Environmental Science.

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 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
D. Kim, Y. Chae, Konkuk University; Y. An, Konkuk University / Department of Environmental Heath Science

Plastic debris is of concern in aquatic ecosystems. 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 than 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
S. Rehse, Leibniz-Institute of Freshwater Ecology and Inland Fisheries / Ecophysiology and Aquaculture; S. Seidensticker, University of Tuebingen / Center for Applied Geosciences; J. Wicht, University of Tuebingen; C. Huhn, University of Tuebingen / Institute of Physical and Theoretical Chemistry; W. Klaos, Leibniz-Institute of Freshwater Ecology & Inland Fisheries; C. Zarfl, University of Tuebingen / Center for Applied Geosciences

Freshwater biota is facing pollution by chemicals, but also by particle materials like microplastics (MP, polymer particles < 5 mm), as well as macroplastics containing 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 trace levels like water pollution. Thus, both their single effects and 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 field-relevant conditions 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 this sorption (immeasurably slow) has determined the 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 (an endocrine disruptor used in contraceptive drugs, and on the amphiphil 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 the single and mixture effects of MP and polystyrene suggest that MP and plastic particles, might have greater capacity to adsorb organic compounds. Polyethylene microbeads are usually floating on the water and are often accumulated in lakes and ponds. Therefore there is a potential for those low nanoplastic concentrations that are not capable of inducing any effect under realistic exposure scenarios however, competitive absorption of pollutants like microplastics (MP, polymer particles < 5 mm). In addition, MP contain a high variety of functional groups which have been shown to affect the plastic properties. 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: 02WK15378). 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 rams horn snail (Marisa cornuarietis) and in brown trout (Salmo trutta f. fario). We examined the effects of the embryo toxicity, the moment of demarcation of 2 µm polystyrene particles, these were non-toxic to adults Daphnia. Here I assessed the uptake and excretion of 15µm microplastic (polystyrene) beads on the model freshwater aquatic crustacean Daphnia magna. Epifluorescence 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 to mortality of Daphnia over time.

TH194  Size matter? Physical effect of large microplastic particles (Polystyrene) on Daphnia magna

R. Aliabadi, University of Reading / Biological Science; A. Callaghan, University of Reading

Microplastics are present in many commercial products such as toothpaste, facial cleanser and body scrubs. Plastics transferred from wastewater treatment plants to surface water end up polluting both marine and freshwater habitats. Several recent studies have shown that these particles vary in size and shape (Napper et al., 2015). My project aims to investigate microplastics’ effects and potential consequences. To do this, I examined the toxicity of polystyrene microplastic in Daphnia magna and Daphnia pulex using a standardized toxicity test. I exposed D. magna to different concentrations (50-1000 µg/L) for 96h. These microplastics are much smaller than the single effects of microplastics on aquatic invertebrate species. 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 microparticules 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

B. Camey Almroth, Goteborg University / Department of Biology and Environmental Sciences; T.M. Karlsson, University of Gothenburg/ J. Näslund, University of Gothenburg / Department of Biological and Environmental Sciences; B. Håkansson, University of Gothenburg / Department of Marine Sciences

As plastic production increases worldwide, reaching over 311 million tons in 2015 (PlasticsEurope, 2015), 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. In this study, two different rivers, the first receiving industrial waste water from factory site, and the second receiving storm water, were investigated. These storm water samples were analyzed for plastic fine particles (PFPS) using the Vybrant® DyeCycle™ Green Uptake Assay Kit. The PFPS were characterized by an increase in fluorescence intensity in Daphnia magna test, which indicated that microplastics carried from stormwater into aquatic environments can have detrimental effects on freshwater organisms. This study is an important step towards understanding the potential impacts of microplastics on aquatic ecosystems, and highlights the need for further research to better understand the ecological consequences of plastic pollution.

TH197  Effects of polystyrene microplastics in brown trout (Salmo trutta f. fario) and the giant rams horn snail (Marisa cornuarietis)

H. Schmieg, Tübingen University / Animal Physiological Ecology, S. Krais, University of Tübingen / Animal Physiological Ecology; H. Köhler, University of Tübingen / Animal Physiological Ecology; R. Triebborn, University of Tübingen / Animal Physiological Ecology

Microplastics are of concern in aquatic ecosystem. They can be combined with other particulate materials like microplastics (MP, polymer particles < 5 mm). In addition, MP contain a high variety of functional groups which have been shown to affect the plastic properties.

TH198  Microplastic ingestion by brown trout living in a river polluted by industrial plastic production

B. Camey Almroth, Goteborg University / Department of Biology and Environmental Sciences; T.M. Karlsson, University of Gothenburg; J. Näslund, University of Gothenburg / Department of Biological and Environmental Sciences; M. Hasselöv, University of Gothenburg / Department of Marine Sciences

As plastic production increases worldwide, reaching over 311 million tons in 2015 (PlasticsEurope, 2015), 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. In this study, two different rivers, the first receiving industrial waste water from factory site, and the second receiving storm water, were investigated. These storm water samples were analyzed for plastic fine particles (PFPS) using the Vybrant® DyeCycle™ Green Uptake Assay Kit. The PFPS were characterized by an increase in fluorescence intensity in Daphnia magna test, which indicated that microplastics carried from stormwater into aquatic environments can have detrimental effects on freshwater organisms. This study is an important step towards understanding the potential impacts of microplastics on aquatic ecosystems, and highlights the need for further research to better understand the ecological consequences of plastic pollution.

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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 the tracts using a non-enzymatic method 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.

Do microplastic fibers exert a greater toxicity than microbeads? 500 μm microbeads are used to expose this calanoid in a (semi-)static setup in order to keep microplastic films in contact with the copepod. 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 with different size and concentration. Because of our preliminary data on (1) the influence of MP size (4.5 vs 45 μm Ø) and concentration of BAP (10 nM) on a range of biological endpoints, we hypothesize that the concentration-dependent models are appropriate for the chosen system and endpoints. We can also quantify the relative importance of the different processes involved in the uptake and biological responses of these MPs.

Bayesian analysis of the take-up and effects of microplastic particles in the marine copepod Calanus finmarchicus

The marine copepod C. finmarchicus is a key species in the North-Atlantic and has a high potential for long-distance migration. It is a filter feeder and plays an important role in the marine food web. C. finmarchicus is known to ingest and accumulate microplastics, which can have negative effects on its physiology and ecology. Understanding the mechanisms by which C. finmarchicus interacts with microplastic particles is essential for predicting the impact of microplastics on marine ecosystems.

Materials and methods

C. finmarchicus were exposed to spiked sediments containing microplastic beads and fibers in the ecologically important polychaete worm Hediste diversicolor. For this work was to compare the uptake and toxicity of polystyrene NPls (50 nm) and 3000 μm microbeads and fibers (polyamide/polypropylene/polyvinylchloride) at a size range of 50 μm to 50 mm. The plastic particles were ingested by H. diversicolor filaments. Short-term exposure to polystyrene MPs alone or in combination with Cd (1 µM). This indicated higher capacity of polystyrene to adsorb BAP than metallic (Cd) pollutants. The histological assessment showed that after 1-day exposure, microplastics are found in the stomach, primary ducts, whereas smaller MPs were found in the connective tissue concentration-dependent manner. The larger particles were found in the stomach matrices - the role of particle properties in internalized by mussels depending on their size and that MPs act as carriers for vectors for pathogens and other pollutants present in the water column and lead to their accumulation into the digestive system, disturbing feeding processes, and general purpose polystyrene (GPPS) in both the absence and presence of microalgae. Custom made transparent plankton wheels immersed in a water bath were 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.
feeding in E. marinus.

TH204 Uptake and effects of microplastic particles in the marine copepod Calanus finmarchicus

G. Østrov, Norwegian University of Science and Technology / Department of Biology; D. Altin, BioTrix; B. Hansen, SINTEF Materials and Chemistry; A. Booth, SINTEF Ocean / Environmental Technology; A. Olsen, Norwegian University of Science and Technology; L. Salaberg, NTNU / University Biology

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 copolymer particles (10–100 nm polystyrene low-density polyethylene (LDPE)) and general purpose poly styrene (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 physical, chemical and effects assessment

A. Booth, SINTEF Ocean / Environmental Technology; J. Farkas, S. Jähnen, K. Zählsen, S. Kubiwicz, SINTEF Materials and Chemistry

Globally, car tyres have been identified as one of the most significant sources of microplastics (MPs) particles to the environment. Due to the large production of car 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 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 responses from zooplankton to seabirds. Particulate plastic and fibers were added to fish larvae 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 remains unknown, but may now become more known 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 Mytilus microplastics and organic and metallic pollutants

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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) and concentration (1, 10 and 100 particles/ml) on cumulative effects between ingested MPs and algal prey; 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 midgut, whereas smaller MPs were found in the connective tissue surrounding the digestive tubules and gonia. 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 within 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, particularly, 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 (MPs). In addition, MP particles are manufactured for specific applications in products such as cosmetics and household goods, which may release microplastic debris in the ocean. These MPs can be ingested by invertebrates and cause damage to them. Moreover, occurrence of nanoparticles (NPs, < 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 adsorbed MPs may act as 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 NPs (50 nm) and MPs (0.5 and 4.5 µm), and alone and with adsorbed benzo[a]pyrene (bap), using primary cultures of hemocytes, immune cells of mussels Mytilus galloprovincialis. Marine MP uptake and effects were assessed by respiration test, energy and electron microscopy and by FT-ICR-MS. Ingestion of MPs and NPs to the mussels Mytilus galloprovincialis was assessed using ESI-Q-TOF-MS. Exposure to a wide range of NPs and MPI 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 mm NPs). Exposure to NPs and MPs with adsorbed bait (1 and 158 mM) caused a significant decrease in cell viability and increase in ROS production at the highest bait concentration, especially in the case of 0.5 mm MPs. Overall, results indicate the potential of polystyrene NPs and MPs to cause oxidative stress and to cause ROS production in mammalian 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 IT101-13) and UPV/EHU (UP I11/37 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 biota, and 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. Mussel were exposed to polystyrene (PE) microplastics, of a size (3-12 µm, mean particle size 7.5) comparable to that of microplastics 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 pseudoacute 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 and characteristics 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 2018, 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 polystyrene 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. Proteins identified points 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 the 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 native polyvinyl chloride PVC (MPV) spiked with 0.1% (w/w) of PVC (MP1). 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 µm² 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. Thus, these results demonstrate 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 defense 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.
0.14 items/m³; 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 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 curtilly additive chemicals. Based on previous non-targeted screening areas. Further ecotoxicological investigation on WS skin biopsies will be carried out for a worldwide ecotoxicological risk assessment of this endangered species including plastic additive and gene expression biomarker detection.

TH213

Styrene oligomers (SOs) in polystyrene based consumer products: Tracing major contributor to environmental SOs

Z. Tian, S. Kim, Incheon National University / Department of Marine Science Plastic debris is global emerging issue. The results of recent studies suggest a large uncertainty in the global distribution and composition of plastic debris, due to lack of data depended on plastic particles. SOs can be found 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 shown 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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Plastic debris (PXs) are widely applied to various plastic products as plasticizer and softener. PAEs are not chemically bound to the polymeric matrix but physically mixed; consequently, they can leach, migrate or evaporate into the surrounding environment during their use or after disposal. PAEs have become an environmental concern due to their ubiquitous presence and their potential 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 additives and contaminants, based on scientific studies analyzing plastic debris. 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.

TH215

Weathering of plastic materials and identification of their leachates

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At the ocean surface the plastic is exposed to sunlight, oxidents and physical stress leading to degradation [1]. It is important to identify and assess potential environmental hazards that plastics could be due to uncertainties that are 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

TH216

Investigation of the effect of plastic additives on the Plastic water partition coefficient of selected persistent organic pollutants (POPs) in batch tests


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 these tests have been previously subjected to the size of < 1.500 µm. The samples were analyzed by using High Performance Liquid Chromatography (HPLC). Adsorption isotherm is applied to determine PE-water partition coefficients (Log KPE-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 Kow = 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 KPE-W was observed for the five 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

Dibenzo-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 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. The plastic pellets were sampled 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 - polystyrene (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 subamples 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 material itself, and by analyzing leachates generated in the study areas. Further ecotoxicological investigation on WS skin biopsies will be carried out for a worldwide ecotoxicological risk assessment of this endangered species including plastic additive and gene expression biomarker detection.
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 dichloromethane 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.

**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 (plpLFER) 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 sorbates (e.g., Kow). Poly-parameter linear free-energy relationships (plpLFER) and multivariate linear free-energy relationships (mvLFER) were determined and high correlation coefficients were obtained. The slopes of the mvLFER were higher than the plpLFER. Further experiments are necessary to determine if the same approach could be used for aged polystyrene microplastics to investigate the contribution of individual molecular interactions to overall sorption. The results showed that the development of a plpLFER equation allowed a precise prediction of sorption of organic compounds by aged polystyrene. The contribution of individual molecular interactions to overall sorption of aged microplastics could be characterized using the plpLFER 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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Cadmium bioavailability was assessed by measurement of cadmium from metallothionein, which can be determined by real time RT-qPCR. We first investigated the biofilm growth on PVC MPs (125 – 250 µm diameter) in freshwater obtained from a zebrafish (Danio rerio) husbandry facility after growth periods of 24 h, 96 h and 168 h. The presence of a biofilm on PVC particles was quantifyed 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 mM EDTA. Subsequently, a biofilm was allowed to grow for seven days on PVC particles. Both pristine and biofilm-associated particles (1 G/L) were measured (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 concentration in fish larvae which can be determined by real time RT-qPCR (RT-qPCR) after RNA extraction from whole larvae. We verified our results based on zebrasfish 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 findings (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 mm to 5 mm by FTIR and Raman microscopy**

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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 organic contaminants can be ingested by marine animals, which can result in human exposure to these particles and consequent adverse health effects. Microplastics in the aqueous phase delivers complete and detailed microplastics analysis. Detailed results are given in transference of hydrophobic organic contaminants from different polymers and origin) sampled in the surrounding 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.

**TH222**

**Freshwater sediment microplastic from an urban river in the west of Scotland**

R.M. Blair, S. Waldron, University of Glasgow; C. Gauchotte-Lindsay, University of Glasgow / Infrastructure and Environment; V. Phoenix, University of Strathclyde

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/kg of dry sediment in December 2015 and February 2016, respectively. Secondary MP, namely fibres were predominant, constituting 88–98% 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-fibrous particles falsely 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 A. Naji, Z. Esmailli, University of Hormozgan

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 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, fibres 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 S. Minteng, Utrecht University / Copernicus Institute of Sustainable Development; R. Messina, Copernicus Institute / Utrecht University, P. Bauerlein, KWR Watercycle Research Institute / Analytical and Environmental Chemistry; S. Dekker, Copernicus Institute / Utrecht University; A. Koolman, Wageningen University / Aquatic Ecology and Water Quality Management; A. van Wezel, KWR Watercycle Research Institute / Chemical Water Quality and Health

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 consequently 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 aims 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, pyGc-MS, and atomic force microscopy techniques. The behaviour of polyvinylene 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? L.I. Devriese, ILVO; F. Tack, R. Van Pouchke, Ghent University - Department of Applied Analytical and Physical Chemistry; M. Bossaer, K. Hostens, Flanders research institute for agriculture, fisheries and food (ILVO), Animal Sciences Unit, Aquatic environment and quality; B. De Witte, ILVO / Aquatic environment and quality, J. Robben, Institut Royal des Sciences Naturelles de Belgique - Laboratory for Aquatic Environment and Quality

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 selected 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 with concentrations in the range of 0.65 µg/kg and 0.1 µg/kg respectively. The number of microplastics located between contaminants 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? C.J. Thiele, University of Southampton / Centre for Environmental Sciences, Faculty of Engineering and the Environment; D.N. Coggon, University of Southampton / MRC LifeCourse Epidemiology Unit; S.J. Harrad, The University of Birmingham; M.D. Hudson, University of Southampton / Centre for Environmental Sciences; A.C. Jensen, University of Southampton / Ocean and Earth Science; A.E. Russell, University of Southampton / Chemistry

Microplastics are found in the digestive systems of many marine organisms and consequently 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 systems. 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. In the current study, fish meal from different sources 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. & Ash, 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

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 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 different pressures, such as increased pesticide use, climate change, and 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. This 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 eco-toxicity) and finally an extrapolation strategy for regional upscaling of assessment results.

TH230 Assessment of toxicological thresholds based on legal limits from REACH regulation

M. Tchobanoglous, ADAI-LAETA / ADIA / Department of Mechanical Engineering, University of Coimbra; J. Ribeiro, ADAI-LAETA / Department of Mechanical Engineering; F. Freire, ADAI-LAETA

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 a list of substances, which include hazardous 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 (So_{2}) 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} 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.

TH228 Freshwater ecotoxicity as an environmental impact category in LCA - Findings of a cross-functional taskforce

N. Otte, Henkel AG & Co. KGaA; C. Cooper, International Zinc Association / TH228

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 methods for the inclusion 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 characterise 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 current data availability and ESC’s conservatively projected ingredient volumes. 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. '

TH229 A framework for contaminants in environmental assessment of organic waste recycling in agriculture

A. Avadi, T. Wassenaa, E. Doelsch, CIRAD / UPR Recyclage et risque

Ever-increasing waste production has prompted the need for more sustainable waste management, from economic, environmental and human health perspectives, that is, including the consideration of potential adverse effects in addition to 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 impact 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 different pressures, such as increased pesticide use, climate change, and 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. This 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 eco-toxicity) and finally an extrapolation strategy for regional upscaling of assessment results.

TH236 Determination of the estrogenic potential of water

T. Nagai, Institute for Agro-Environmental Sciences, NARO

In recent years, there has been a growing concern over the potential estrogenic effects of endocrine active substances (EAS) on aquatic organisms. This concern is due to the increasing use of chemicals that can affect the endocrine system of organisms, leading to changes in their reproduction and development. To evaluate the estrogenic potential of water samples, different methods and approaches have been developed. One of these approaches is the use of in vitro tests conducted with aquatic microorganisms, such as the alga Chlorella. This study aimed to develop and validate a novel in vitro test for the determination of estrogenic potential in water samples. The test involved the use of algal microplate assay, which was adapted from the standard method of ISO 13629-1. Samples of water were placed in a special device that maintains a constant temperature and carbon dioxide supply from the air. The toxicity was defined by the difference in cell density and can be determined using a microplate reader. Test performance was assessed using positive (3,5-dichlorophenol and malachite green) and negative controls (water). The results demonstrated the suitability and feasibility of the developed test method for the determination of estrogenic potential in water samples. The test was able to detect estrogenic compounds at concentrations as low as 10 μg/L. Additionally, the test showed good concordance with precision data derived from LC/MS/MS methods. The results of this study indicate the potential of the developed test method for the routine determination of estrogenic potential in water samples, which could be useful for water quality monitoring and risk assessment.

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, due to the fact that specific water quality standards for most contaminants were not available. To study these 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 species sensitivity against their log10-effective concentrations. 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 QLS-like multivariate linear regression model to accurately estimate SSD-parameters for approximately 1500 substances based on five of these characteristics, making use of the ε-Toolbox of the Dutch National Institute for Public Health and the Environment (RIVM).

Eventually, these methodologies may be applied for the assessment of emerging chemicals. Multiple models were trained on acute LC50 and EC50 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-inter-species variation μ has been achieved 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
P. Pandard, INERIS; J. Roembke, ECT Okotoxikologie GmbH; R. Weltens, VITO
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 specifically address the property Ecotoxic. It 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) or for toxicity (in steps 2 and 3) are exceeded or not. Based on experiences of 20 years of waste testing, an assessment 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).

C. Eiden, newdiagnostics GmbH; K. Hettwer, QuoData GmbH; M. Jähne, new diagnostics GmbH; K. Frost, QuoData GmbH; K. Simon, new diagnostics GmbH; S. Uhlig, QuoData GmbH
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 determination of estrogenic activity in different water samples. Thirteen laboratories from four countries analysed the 17β-estradiol equivalents (EEq) in the 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 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) 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 %. 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 waste.

TH234 Novel bioassays using Chlorella and Daphnia for rapid detection of water toxicity
Y. S. Grigorey, Siberian Federal University / Department of Ecology and Environmental Study; T. Shashkova, Siberian Federal University / Department of Ecology and Environmental Sciences; E. Stravinskene, Siberian Federal University Bioindication and bioassay coupled with chemical analysis are widely used for environmental pollution monitoring. Despite many water toxicity bioassays becoming more rapid and simplified, the results are not always in agreement and are not suited to emergency 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 600 000 cells per 24 hours when algae 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 equal 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 toxics 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
T. Nagai, Institute for Agro-Environmental Sciences, National Institute of Agro-Environmental Sciences
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: Rhizophyllum brooksia (Chitridiomycota) strain NBRC 103829, Chrytromyces hyaluns (Chitridiomycota) strain NBRC 102555, Tetracaulon 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: Inhibition of filamentous fungi method). Toxin concentration is to be known which 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
S. M. Robbins, new diagnostics GmbH; K. Frost, QuoData GmbH; K. Simon, new diagnostics GmbH; K. Simon, new diagnostics GmbH; D. van de Meent, Radboud University / Department of Environmental Chemistry; E. Stravinskene, Siberian Federal University Bioindication and bioassay coupled with chemical analysis are widely used for environmental pollution monitoring. Despite many water toxicity bioassays becoming more rapid and simplified, the results are not always in agreement and are not suited to emergency 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 600 000 cells per 24 hours when algae 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 equal 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 toxics 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

SETAC Europe 27th Annual Meeting Abstract Book
water test systems (OECD 309)

S. Höger, Innovative Environmental Services (IES) Ltd / Environmental Toxicology; C. Wijntjes, W. Völkel, D. Adam, Innovative Environmental Services IES Ltd

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 compounds patterns 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 Count Data from Ecotoxicity Studies

U. Green, DuPont / Dynamic Simulation, Control, and Optimization

Quantal data is a type of count data that often arises in ecotoxicity studies. A fixed number of subjects are exposed and the interest is in the number of those subjects that respond, e.g., die or develop a specific type of lesion or condition. There are 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 Oao-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. GLM 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 residuals that use normal or binary error structure that can 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

U. 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 is in 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, generalized-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 simulation studies and through application to real 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 effect estimation, confidence intervals 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 genes 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 effect no effect concentration (ECx) case by case. However, scientists generally have to choose the “non-toxic” concentration (Nic) 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 a method for choosing a chemical concentration which can reasonably 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 Nic 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 Luminescencebacteria testing in natural samples - inter laboratory comparison test

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Luminescence 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 dispensed into typical sample containers containing sediment and/or water in a lab industry process water. Finnish Environment Institute SYKE organized an interlaboratory comparison test providing a coloured samples extracted from creosote contaminated sediment a addition to an artificial water samples spiked with 3,5-dichlorophenol. For the statistical analysis of the results, the mean or robust measures from 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 D%-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 difference from of EC50 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 EC50 values than standard method in both cases. The nature of the sample was suitable for comparison of kinetic luminescence test to the standard method. However, the heterogeneity of normal samples was 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 peak 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 peak particle size. A factorial design was used to experimentally determine 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. The peak was sieved to obtain the small (≤1 mm), the medium (1 mm ≤ x ≤ 2 mm), and the big (2 mm ≤ 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, PPS 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. 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 availability (BARGE and EN 14997) (iii) and leaching tests for ecotoxic availability (OECD nr 29 and EN 14991). A set of twelve waste water samples and model 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 of relatively non-hazardous elements (e.g. 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 must be performed in a test method that is scientifically sound and practical. The assessment of biodegradability in a test method that is scientifically sound and practical choices. This approach has important risk assessment implications and it is important to choose the right test method. To overcome this ambiguity, we propose an algorithm for choosing a test method that is scientifically sound and practical. The algorithm is based on a large database of regulatory guideline studies. The choice of test method is based on several factors: (i) the statistical difference from effect zero or the control and (iv) the probability of obtaining 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 used. The algorithm is based on a large database of guideline studies the relative merits of these alternative approaches in order to make scientifically sound and practical choices.

**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


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 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. One product family can contain several, approved biocidal products that cover the same or similar properties 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 parameter (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. The 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 harmful 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 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 methodology 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.
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, the intensity of spiking may not expect substantial temporal and spatial dynamics of local concentrations, especially in the vicinity of the interface between 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 different test conditions. As result, different EC50 and LC50 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 EC50 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 POP 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 concentrations of the respective endpoint. The results were 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 because 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 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 endorsed and released by the USEPA, the US Geological Survey, and US Army Corps of Engineers to allow environmental professionals to perform Tier 2 environmental risk assessment with further data on the ecotoxicological effects of a compound, without performing toxicological tests with the midge the 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 revision of this database that inform regulatory scientists and methodologists 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 Leptochirus.
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There has been considerable research in recent years to improve tests for Hyalella, Chironomid, and Leptochirus, 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, in particular 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 Leptochirus phaeothisis 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 organisms should be fed during the test. We also have data to support that the lessons learned from Hyalella chronic study should be applied to Leptochirus, 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, it is 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 (HCS) 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 RACsed). 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 into sediment and may persist there, making sediment toxicity tests most appropriate. The aim of the present study was to generate a Tier-2 RACsed and compare this with the previously obtained Tier 1 RACsed and Tier-3 RACsed. 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 an SSD derived from a Tier-2 RACsed value. Valid 10 d sediment-spiked tests were performed with the benthic arthropods Chiromus dilutes, Chiromus riparius, Caenis sp., Ephemera danica, Sialis lutaria, Hyallela 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/g OC together with an AF of 15, resulted in a Tier-2 RACsed value of 0.12 μg/g OC. This Tier-2 RACsed seems to be protective and worst-case when compared with the Tier-3 RACsed of 0.263 - 0.395 μg/g OC derived from the outdoor sediment-spiked microcosm test. In addition, this Tier-2 RACsed was more than a factor of 2 higher than the Tier-1 RACsed (of 0.049 μg/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 evaluation
M. Cusido-Martinez, Centre Ecotoxic; L. Mendez, UPV/EHU / Zoology and Cell Biology; M. Wildi, Swiss Centre for Applied Ecotoxicology EawagEFPFL; R. Kase, Swiss Centre for Applied Ecotoxicology EawagEFPFL; B. Ferrari, Ecotoxic Centre CH; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy Physiology and Cell Biology.

The development of a standardised quality control 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 standardised 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 reporting ecotoxicity data (CRED), which 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 the 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
Variation of perfluoroalkyl substances in water, sediment and fish samples from Ebro Delta (Catalonia, Spain)
M. Llorca, IDAEA-CSIC / IDAEA; E. Pinotti, Bologna University; G. Casas, IDAEA CSIC Barcelona; A. Tellbucher, University Duisburg-Essen; D. Almeida, Universidade de Lisboa; E. Griony, E. Dimelli, University of Bologna; M. Farre, IDAEA-CSIC / Environmental Chemistry; M. Darcelo, IQOAC-CSIC / Dept Environmental Chemistry.

The contamination of the aquatic environment by perfluoroalkyl substances (PFASs) becomes a problem since they are easily transferred between environmental compartments reaching groundwater, soils, sediments and biota. However, the partitioning of these compounds 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 sewage 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, perfluorooctanoic acid and perfluorooctane sulfonate were the most commonly used and concentrated in water samples. The most contaminated samples were influent and effluent WWTPs where perfluorooctanoic acid showed the highest concentrations in all sampling campaigns (100 to 2000-time greater than other water samples). In the case of sediments, perfluorooctane sulfonate (PFOS) was the compound detected at higher concentrations (>100 μg/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 dominant compound was PFOS despite its high production concentrations (<1 0.2 μg/g dw). PFOS was always detected in fishes. Waters values agreed with its high persistence, bioaccumulation and biomagnification. On the contrary, river fishes showed higher PFAS concentrations (2PFAS from 63.76 ng/g ww to 938.21 ng/g ww). In addition, perfluoralkyl carboxylic acids were more concentrated than sulfonates. The bioaccumulation factor calculated for freshwater fishes denoted the high capacity of perfluorohexanoic acid, PFPOA, perfluoroctanoic acid and PFOS to be accumulated in fish from the surrounding waters.
analysis of EOCs.

MOPC10
Fate and transport of perfluoroalkyl acids (PFAAs) in soil and groundwater D. Muszbek, LTH, Lund University / Water Resources Engineering; L. Ahrens, Swedish University of Agricultural Sciences and Assessment; K.M. Persson, LTH, Lund University / Department of Water Resources Engineering; R. Berndtsson, LTH, Lund University

PFAAs are compounds that are known for their persistence in the environment and are now under increased scrutiny due to concerns over their potential impacts on human health and the environment. This presentation will provide an overview of the current understanding of the fate and transport of PFAAs in soil and groundwater, with a focus on the importance of understanding these processes for effective risk management and protection of environmental and human health.

MOPC11
Electrochemical treatment of perfluoroctanoic acid and perfluorooctane sulfonate: application to groundwater treatment A. Urtiaga, Universidad de Cantabria / Dep. Ingenierías Química y Biomolecular; C.P. Higgins, Colorado School of Mines / Department of Civil Environmental Engineering; M. Stainton, Colorado School of Mines / Department of Civil and Environmental Engineering Department; C.E. Schaefer, CDM Smith Inc.

The use of electrochemical technologies for the treatment of groundwater contaminated with PFAAs has gained significant interest in recent years. This presentation will discuss the application of electrochemical treatment to groundwater contaminated with PFAAs, with a focus on the use of perfluoroctanoic acid and perfluorooctane sulfonate as model compounds. The presentation will highlight the advantages and challenges of using this technology for the treatment of groundwater contaminated with PFAAs, with a focus on the need for further research and development.

MOPC12
Best Environmental Practices (BEP) guidance for the AFFF, textile and related Fluorotechnology industries S. Korzeniewski, BeachEdge Consulting, LLC; T. Cortina, The Fire Fighting Foam Coalition; K. Schubert, The Chemours Company; F. Adamsky, Duinin 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 determine the potential environmental impacts of their use of AFFF and to reduce these impacts. The presentation will provide an overview of the guidance and provide examples of how it can be applied in practice.

MOPC13
New Mechanisms in Assessing and Managing Per- and Polyfluoroalkyl Substances? Current Needs and Our Recommendations Z. Wang, Swiss Federal Institute of Technology / Institute for Chemical and Bioengineering; I. Cousins, Stockholm University / Department of Environmental Science and Analytical Chemistry (ACES); K. Hungerbühler, M. Scheringer, ETH Zurich / Institute for Chemical and Bioengineering

Per- and polyfluoroalkyl 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 in place to reduce exposure to them on the national, regional or global scale. However, despite extensive testing and regulation, some PFASs are still found in the environment. The presentation will provide an overview of the current understanding of the mechanisms of PFASs and discuss the current needs for the assessment and management of PFASs.

Fate, risk assessment and management of natural toxins: state-of-the-art, challenges and future perspectives (PC)

MOPC14
Plant toxins as groundwater contaminants - do we need to care? H. Hansen, University of Copenhagen / Department of Plant and Environmental Sciences; F. Clausen-Kaaes, University of Copenhagen; B.W. Strobel, University of Copenhagen / Plant and Environmental Sciences; L. Rasmussen, Metropolitan University College; N. Cedergreen, University of Copenhagen / Department of Plant and Environmental Sciences; S. Hansen, University of Copenhagen

Plants produce a wide 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 groundels, paquirolide 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 hectare. These are released from plant to soil via wash-off, or from roots and plant litter, thereby constituting a continuous non-point source of these toxicity risks. Groundwater depletion is a major threat for the safety of these toxins. These are readily decaled to 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 glucosides 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 paquirolide from bracken. All are glucosides, but while the first two groups of compounds are toxic due to their primary degradation products (hydrocyanide and isothiocyanates), paquirolide is the direct toxin (carcinogen) after activation. While groundwater monitoring data for natural toxins are generally lacking, first measurements for paquirolide 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 Phytotoxins Database for Environmental Risk Assessment (PC)
F.E. Gerguson; C. Agroscopio; J. Hollender, Eawag / Environmental Chemistry; M. Scheringer, K. Hungerbühler, ETH Zurich / Institute for Chemical and Bioengineering; T. Bucheli, Agroscope ART / Environmental Analytics Natural Resources and Agriculture

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. 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 organs, as well as the difficulties of the grouping of morphological, geographical, and taxonomical parameters. The review of the literature shows that phytotoxins in samples 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  
B W Strobel, University of Copenhagen / Plant and Environmental Sciences  
The globally occurring bracken fern (*Pteridium sp.*) is widespread in the boreal 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 number of components of individual toxic plants are often not identified. Of which the compound ptaquiloside (PTA) is the most abundant. Ptaquiloside has been shown to be highly water soluble, leachable from bracken foliage 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 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?  
X Jiang, University of Copenhagen; H.C. Hansen, University of Copenhagen / Plant and Environmental Sciences; N. Cedergreen, University of Copenhagen / Department of Plant and Environmental Sciences  
Saponins are natural surfactants, found abundantly in various plant species. Saponin-rich extracts from quillaja and quinoa have been registered by US EPA as acceptable material for control of myrtle rust. To use saponin-rich biopesticides, it is important 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 (HC95), 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. HC95 of quillaja saponins, tea saponins and quinoa saponins were 9.17, 0.54 and 38.59 mg saponin/L, respectively. There is a big disparity 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 populations of water organisms from chemical stress. EQS of these saponins are 0.92, 0.05 and 3.42 mg/L, respectively. However, the application of this minimal concentration factor to the use 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  
E. Skouri-Stathaki, F. Clauson-Kaas, University of Copenhagen; K.K. Brandt, University of Copenhagen / Department of Plant and Environmental Sciences; L Rasmussen, Metropolitan University College; H. Hansen, University of Copenhagen / Department of Plant and Environmental Sciences  
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 pscleusenolide ("the ptaquiloside group"). The group of substances, 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 sorted 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 soil and sediment may act as an indication 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 microbiobly degraded. Soil materials having very low microbial activity such as raw aquifer materials could accommodate longer half-lives, 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 Toxicity 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’s 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/hoosari) and China (juecai/juecan). Different species are used in the trade 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 the bracken in Europe can be imported from China, even past presence of ptaquiloside 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.

**MOPC20**  
Mobility of metallic (nano)particles in leachates from landfills containing waste incineration residues  
D.M. Mitrang, Eawag - Swiss Federal Institute of Aquatic Science and Technology / Environmental Risk and Management; K. Mehrabi, Y. Arroyo Rojas Dasilva, Empa Swiss Federal Laboratories for Materials Science and Technology; B. Nowack, EMPA  
In situ measurement of nanoparticles (PC)  
Waste incineration residues 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 the bracken in Europe can be imported from China, even past presence of ptaquiloside 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.
landfills. Emitted leachates contain a myriad of solids 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 washing, using tap water and deionized water (DI), was performed 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 nanoparticles or contaminants (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 (<100 nm) particles and a high fraction (0.1–0.4) of coarse particles. In our 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

R. Skene, NERC Centre for Ecology and Hydrology / Ecotoxicology; M. Matzke, Centre for Ecology and Hydrology (NERC) / Molecular Ecotoxicology; L. Lynch, University of Birmingham / Geography Earth Environmental Science; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology

A concerted research effort over the last decade has enabled us to significantly improve our understanding of the core chemistry of engineered nanoparticles (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 (cytochrome) displaced the citrate coating immediately upon exposure to an exogenous analyte but led to their stability at low analyte concentration. On the other hand, interacting molecules (tannin 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 more detailed information on ENM surface chemistry, 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

S. Pereira, University of Aveiro / Department of Biology; D. Boyle, Plymouth University; A.J. Nogueira, University of Aveiro / Biology; R. Handy, University of Plymouth; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry Modeling of nanomaterials, nano-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 matrices. The employment of 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 conventional ICP-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 chironomus (Chironomus riparius) at 14A3/37 ng Ag/embryo in choriornates (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.

MOPC3 Mapping of accumulated YRbHITC-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 on the influences, especially based on the organisms in natural environments. For the purpose, plants are good study model since they are exist 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, YRbHITC-doped silica nanoparticles, which have rare earth element of yttrium (Y) and rodamine B isothisiocyanate (RbHITC) dye doped in the core of silica nanoparticles, were synthesized, and after being 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.

MOPC4 Quantification of pesticides in fungi and midge larvae in the presence of nanoparticles

J. Wicht, C. Lorenz, University ofTuebingen; E. Früh, University of Tuebingen / Institute of Evolution and Ecology JD Botany; L. Guluzada, University of Tuebingen / Center for Applied Geosciences; H. Köhler, University of Tuebingen / Animal Physiological Ecology; S. Dietz, University of Tuebingen / Institute of Evolution and Ecology JD Botany; S. Haderlein, University of Tuebingen; C. Huhn, University ofTuebingen / Institute of Physical and Theoretical Chemistry 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 micro-organisms 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 thiaceclorip on the midge larvae of Chironomus riparius were investigated. The food authorities are advised to take these findings into consideration.

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 reasons 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 similar 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.

TUCP02 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 high. We therefore began to explore the reasons and factors underlying 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.

TUCP03 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), chlorpyrifos (EC), spinosad (SC), imidacloprid (SL), dimethoate (EC), bifenazate (EC), fluvalinate (EW), and chlorantraniliprole (SC). The endpoint of the laboratory tests was red mason bee mortality after 48 h of their topical exposure. Also, LD₅₀ 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 survival and the brain of exposed bees. Bifenazate and chlorantraniliprole were the most selective to the bees.

TUCP04 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 ICCPR Non-Api working group a start was made to develop a first-tier acute contact test for Osmia spp. bees. Based on the honeybee protocol (OECD 439) the team designed experiments which we performed to determine the toxicity of dimethoate as test substance, Osmia bicolor, Osmia cornuta were housed in groups and fed 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. bicolor 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. bicolor, respectively. Indicating that a validated and workable test guideline is within reach.

TUCP05 Differentially expressed genes on brains of africanized Apis mellifera exposed to thiamethoxam

P. Decio, UNESP Universidade Estadual Paulista Júlio de Mesquita Filho; T. Roat, UNESP Universidade Estadual Paulista Júlio de Mesquita Filho / Biology; K. Derecka, R. Stoger, University of Nottingham; M. Soller, University of Birmingham; O. Malaspina, UNESP Universidade Estadual Paulista Júlio de Mesquita Filho / Departamento De Biologia, Centro de Estudos de Insetos Sociais (SC), fluvalinate (EW), and chlorantraniliprole (SC). The endpoint of the chlorpyriphos (EC), spinosad (SC), imidacloprid (SL), dimethoate (EC), bifenazate assay design assumes that a 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 xenobiotics 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.

TUCP06 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 resources can aid in the assessment of both single rule stressors and interactions with multiple stressors (e.g. disease and Varroa mites).

TUCP07 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 utilisation and the scientific literature to establish approximative 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 lepidopterans. Furthermore, we assessed the pesticide exposure of 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.
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

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 the UK. In this study, fitting a quantile regression was used for deriving Zn concentrations higher than 287 µg 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 µg l⁻¹ bioavailable zinc, and sites with exposures of greater than 287 µg 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 µg 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 µm) 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
C.E. Schiekag, NIPERA; G. Merrington, WCA Environment Limited; D. Leverett, WCA-Environment Ltd; A. Peters, wca

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-normalised data from chronic laboratory ecotoxicity tests into a Species Sensitivity Distribution (SSD). According to ecotoxicology, 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⁻¹ was established. In other jurisdictions such as 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 regard 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 identify specific differences among databases associated with Ni and 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 criterion maximum load or toxic potential that a chemical could support in 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-specific 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
P. Huntsman-Mapila, Natural Resources Canada; E.R. Garman, NIPERA / Ecotoxicologist; J. Skeaff, R. Beaudoin, Natural Resources Canada / CanmetMINING

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 3.5% respectively. Results of 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 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 reveals that Ni can be removed after 96 h. However, the efficiency of most of the contaminants is limited to those that are removed at the beginning of the experiment. The T/D model 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 A. Maahamoun Ahmed, Université Savoie Mont Blanc; E. Lyauté, Université de Savoie; A. Dubrin, Irstea Lyon-Villeurbanne / UR MALY; C. Bonniveau, J. Gahou, B. Motte, C. Rosy, B. Volat, Irstea Lyon-Villeurbanne; S. Pesce, Irstea Lyon-Villeurbanne; B. Lezchastel. Microbial ecology of anthropotrophic river sediments. 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 mixed exposure, the effects were similar or higher than those evoked 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 C.M. Holmes, R. Vamshi, Waterborne Environmental, Inc.; N. Maples-Reynolds, Waterborne Environmental; J.A. Davies, PCPC (Personal Care Products Council) / Science; B. Jonas, PCPC Personal Care Products Council / Science; S.D. Dyer, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization 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 these models is associated with 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 the 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 (Gouni et al 2011) and combine with individual facility population served and flow estimates using the iSTREEM model. We can then 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 M.A. Karaslan, University of Ege; H. Parlak, M. Boyacıoğlu, A. Akal Arslan, G. Gilseler, S. Tez, B. Nabaltbar, Ege University 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-μg/l. Despite the high degradation in aquatic environment and the wide distribution of parabens in water bodies, they 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 literature on the effects of parabens aquatic organisms while there are researches on tests animals and human cell lines. In the toxicity tests for methylparaben, EC₅₀ values were calculated at 1.25 μg/L for rainbow trout (Oncorhynchus mykiss) were exposed for 48 hours, 91 mg/L for green algae (Pseudokirchneriella subcapitata) that were exposed for 72 hours; 59.9-96 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 risk about the endocrine disrupting effects since the presence 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 chemicals, persistent 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 embryo toxicity test higher concentrations of methylparaben lead to increased of larval abnormalities compared with control group.

TUPC16 Dispersion, fate, and toxicity of aged titanium dioxide nanocomposite-based sunscreens in aqueous environments D. Slomberg, Aix-Marseille Université; J. Labille, CNRS; J. Hubaad, Helioscience; A. Lopes, Laboratoire CNB; A. Tagliati, Heriot-Watt University; S. Hennige, University of Edinburgh; T. Lammel, INIA; J. Jellinek, G. Tsoukatou, J. Sturve, University of Gothenburg In vitro Development and functional characterization of 3D spheroidal aggregate cultures of the rainbow trout liver cell line RTL-W1 Cultures are viable (positive alamarblue staining) and may have different properties recruiting and binding more cells to their surface. After 4 days relatively compact formation and growth. Cell clusters increased in size in the following days by 3.8-fold. Cell morphology varies from highly-compact to more dispersed arrangements. RTL-W1 cultures are able to form spheroidal aggregates. RTL-W1 cell line has been selected because of its suitability to predict the toxicological impact of contaminants on natural sediment communities and to improve risk assessment of metal contamination.

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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 a coral larvae assay (screening box, 

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In the European Union and Switzerland, 26 organic compounds and one inorganic (titanium dioxide TiO2) compound are registered as UV filters. Some of the organic UV filters have shown hormonial activity in vivo. On the other hand UV filters may 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-TiO2 and organic UV-filters in activated carbon or precat 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 Kátzensee. To monitor the inorganic UV filter TiO2, water samples were collected and filtered through a 0.4 μm cellulose acetate membrane. After the filtration, the filter samples were digested with nitric acid 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 follow: 2-Ethylhexylsalicylate (EHS), 2-phenylbenzimidazole-5-sulfonic acid (PMDSDA), Avobenzone (AB), Ethylhexyl methoxycinnamate (EHMC), Octocrylene (OC) and Oxbenzone (OB). In the swimming pools and the compensating reservoirs Ti concentrations varied between 1 and 9 μg/L. After the precat filter Ti concentrations were in the range of below limit of quantitation (LOQ) to 0.8 μg/L. The precat filter acts as an efficient physical barrier for nano-TiO2 particles. According to results, parallel to the fate of the precat filter, the activted carbon filter was 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: monocostituents, multicoitnstituents, & UVVCs. Across these substances type several families always present a challenge to test especially in ecotoxicology and environment. One of these challenging groups is fragrances. In fragrance chemicals fall under multiple categories, they can be natural, synthetic, monocoitnstituent, multicoitnstituents or considered as UVVCs. One group of fragrances that falls under the title of multicoitnstituent/UVVC 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, resinsoids 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 ecotoxic testing: they are (mostly) solid(s), extremely viscous resins, with a frozen honey-like texture. Last year we presented protocol and results from a set of OECD screening tests on both samples [1], and we found no adverse effect below 100μg/mL (loading rate). However, some effects appeared at the highest loading rate of 1000 μg/mL. 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 on present and discuss these results on their ecotoxicity properties and the conclusion that we can draw from such results: can these terpene-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.
S. Wieck, O. Olsson, K. Kämmerer, Leuphana University Lüneburg; U. Klaschka, University of Applied Sciences Ulm / Sustainable Chemistry and Material Resources

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. 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 25 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 contain 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. These results 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 spherical aggregate cultures of the rainbow trout liver cell line RTL-W1
L. Lamell, INIA; J. Jellinek, G. Tsoukatou, J. Sturver, University of Gothenburg

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 retain certain tissue-specific functionalities when they are grown as three-dimensional (3D) spherical aggregate cultures. Our objective is to develop and functionally characterize 3D spherical aggregate cultures based on the rainbow trout liver cell line RTL-W1 for environmental toxicology 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-W1 cells share morphological and functional characteristics with bile preductural 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 spherical aggregate cultures when cultured in suspension culture plates under continuous movement on an orbital shaker. Spherical formation was initiated via collision of single cells resulting in foci. They allow the generation of foci of up to 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 spherical 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 hepatocytic-specific genes, and response to chemical exposure. Preliminary results suggest that RTL-W1 cells grown in three-dimensional spherical aggregate cultures are viable (positive alamarblue staining) and may have different properties compared to those grown in conventional monolayers.
consulting; B. Marks, WCA Environment Ltd

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 available already data vs. the REACH testing requirements, with the aim to minimize the need for vertebrate testing (cfr. 3Rs in chemical risk assessments).

Colin May took an early decision 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 in 2016. Risk RAFF 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 RAFF 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

S. Schmutz, Swiss Federal Institute of Technology / Environmental Sciences; M. Chrien, Swiss Federal Institute of Technology / Health and Safety Executive; C. Beata, Chemicals Regulation Directorate; R. Bensted, Fera Ltd.; J. Handley, Envigo; T. Hutchinson, School of Biological Sciences, Plymouth University / School of Biological Sciences; K. Ryder, UK Home Office; D. Schehan, Cefas; J. Snape, AstraZeneca UK Ltd. / AstraZeneca Global Environment; G. Whale, Shell Health & Safety; J. Wheeler, Dow Agrosciences; R. van Egmond, Unilever; N. Burden, NCIRs

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 Ecotoxicity Working Group, initiated by the UK National Centre for the Replacement, Refinement and Reduction of Animals in Research (NCIRs) 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) potential test read-across opportunities; (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 ecotoxicity prediction tool for pharmaceuticals in environmental risk assessment

A. Hirose, NIH, Tokyo, Japan; M. Kurimoto, National Institute of Health Sciences / Division of Risk Assessment; H. Shiraiishi, National Institute for Environmental Studies; H. Yamamoto, National Institute for Environmental Studies / Center for Health and Environmental Risk Research; N. Tatarazako, National Institute for Environmental Studies / Environmental Risk; T. Nunomura, Tokyo Heisei University / Pharmaceutical Sciences; T. Yamada, National Institute of Health Sciences

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 medicine 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 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. 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 computed 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 tend to be higher than measured values in hydrophobic pharmaceuticals with lower octanol-water partition coefficient. The NOEC values of 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 ecotoxicity 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

J. A. Arnot, WEPC01 / WEPC03 / PETA International Science Consortium Ltd., London, UK; D. Wenger, EMPA; P. Scheeder, University of Zurich; B. Nowack, EMPA / Environmental Sciences / Environmental Risk; T. Nishimura, Teikyo Heisei University / Pharmaceutical Sciences; T. Yamada, National Institute of Health Sciences / Pharmaceutical Sciences; T. Nagamune, Teikyo Heisei University / Pharmaceutical Sciences; T. Yamada, National Institute of Health Sciences

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 (NCIRs) 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) potential test read-across opportunities; (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.

**TUPC25**
Reducing the number of fish used in acute toxicity testing: Incorporation of the Fish Embryo Acute Toxicity test into the threshold approach


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. Several alternative models of aquatic toxicity are available, such as the OECD 401 in vitro test, known as the in silico ecotoxicity prediction tool for environmental chemicals, and

[1] T. Y. Sun, N. A. Bornhöft,....
totoxicity 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 with 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 the methodology 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 acceptability 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.

**WEPC01**

Adverse effects of particles: 2017 state-of-the-art of engineered nanoparticles as a platform for micro-nanoplastics research

A. Jemec, University of Ljubljana, Biotechnical Fac. / Department of Biology; D. Kühnel, Helmholtz-Centre for Environmental Research / Biomedical Ecotoxicology

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).” Even 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 microscopical 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 toxicity 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 role 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

D. Wenger, EMPA; P. Scheeder, University of Zurich; B. Nowack, EMPA / Technology and Society Lab

Evaluating the magnitudes of flows and concentrations of anthropogenic pollutants in different domains of any society is necessary to assess human impact on the world. 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 could 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 flow 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 available 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 actors of plastics flows on a chemical basis and to compare them. In the future, the model will be used to identify potential control points for reducing the plastics flows in society.

**WEPC03**

A potential for knowledge transfer: parallels and differences in aquatic ecotoxicology between engineered nanomaterials and micro- and nanoplastics

S. Scholz, Helmholtz Centre for Environmental Research / Department of Environmental Engineering; A. Baum, DTU Environment / DTU Environment; N.B. Hartmann, Technical University of Denmark (DTU) / DTU Environment Research over the past few years has shown that microplastics (< 5 mm) are widespread in aquatic ecosystems worldwide. The continuous fragmentation of plastics with age results in nanoplastics that 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. Identical or different test systems for the same 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

A. Praetorius, T. Hüffer, University of Vienna / Department of Environmental Geosciences; S. Wagner, Helmholtz Centre for Environmental Research GmbH - UFZ / Department of Environmental Geoscience; F. Von der Kammer, Vienna University / Department of Environmental Geosciences; T. Hofmann, University of Vienna / Department of Environmental Geosciences

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 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 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 particular nature of both engineered nanoparticles (ENPs) and plastic particles, it quickly becomes apparent that there are some striking parallels 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 particle nature. Consequently, fundamental particle transport processes, described by colloidal 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.**

M. AL-SID CHEIKEH, University of Plymouth / Marine sciences and engineering; R. Kaege, Eawag Swiss Federal Institute of Aquatic Science and Technology; S.J. Rowland, Plymouth University; R.C. Thompson, Plymouth University / School of Mechanical Engineering Sciences and Environmental Systems Engineering; A.J. Meakin, Plymouth University / School of Environmental Sciences and Engineering

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%), forces are controllable within small volume of 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) contribute to work at environmental nanomaterial concentrations and thus (iii) contribute to numerous studies that need these accurate measurements such as nanotoxicology studies that aim at assessing the risk of nanoplastics in the environment. Baalousha M, Prasad A, Lead JR (2014). Environ Sci Process Impacts 16:1338–1347. doi: 10.1039/C4EM00712J

**WEPC06**

**Uptake of differently sized polystyrene nanoparticles by D. magna and influence of protein corona as a function of presentation mode**

F. Nasser, University of Birmingham / Geography Earth and Environmental Science; I. Lynch, University of Birmingham / Geography Earth Environmental Science

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. In aquatic ecosystems, these materials have found their way to the environment, and the potential for NPs to adsorb toxic 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 size or adsorbed contaminants. Studies of the uptake and adsorption of NPs by organisms 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 carried out in the determination of NP sizes (50 and 500 nm) under various presentation modes such as bare exposure, feeding during excretion and being incubated with a food source algae Chlorella 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 forth the importance in assessing bioaccumulation.

**WEPC07**

**Synthetic textiles as a source of microplastics from households**

D.M. Mitrani, Eawag - Swiss Federal Institute of Aquatic Science and Technology / Environmental Risk and Management; E. Hernandez, Emпа, Swiss Federal Laboratories for Materials Science and Technology; B. Nowack, EMPA

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. Fibres 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. Triplate samples of 2 synthetic textiles made of 100% fibers were knitted into two different weaves (interlock and jersey) and washed under three different conditions (washing day, detergent products, 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 > 3000) to measure (i.e. distribution and total mass) the release from 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 fabric fibers and 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 textile 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**

I. Bartolozzi, F. Rizzi, M. Frey, SantAnna School of Advanced Studies / Institute of Management

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. Within 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. 3. Results and conclusions The results show good agreement within each region is unclear and country-level data is insufficient for detailed calculation of greenhouse gas savings of biofuels compared to their fossil pendants. 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. Fibres 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. Triplate samples of 2 synthetic textiles made of 100% fibers were knitted into two different weaves (interlock and jersey) and washed under three different conditions (washing day, detergent products, 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 > 3000) to measure (i.e. distribution and total mass) the release from 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 fabric fibers and 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 textile manufacturers choosing a different textile weave when possible or recommending different laundering conditions to minimize the release of MP fibers from products.
bioassay scenario has the highest LCC value due to the high annual maintenance and biomass fuel costs, especially if pellet biomass is used. The 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.\nu

**WEPC09**

Methodological implications on the greenhouse gas balance of 2nd generation biofuels - ISO vs. RED (2009/28/EC)

1. Rodríguez-Serrano, I. Herrera, D. Gárrain, C. De la Rúa, N. Caldés, Y. Lechon, CIEMAT / Energy Dept Energy Systems Analysis Unit

**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: photovoltaic (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).

**WEPC10**

Sustainability assessment of hybrid concentrated solar power/biomass mini power plant

I. Rodríguez-Serrano, I. Herrera, D. Gárrain, C. De la Rúa, N. Caldés, Y. Lechon, CIEMAT / Energy Dept Energy Systems Analysis Unit

**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: photovoltaic (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 energy transition. 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 result of material needs 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 additionally 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 energy transition from 2012 to 2050, induce more than 4.4 million tonnes of CO2-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

C. Oberschelp, ETH Zurich; S. Pfister, ETHZ / Chair of Ecological Systems Engineering; S. Hellweg, ETH Zurich / Institute of Environmental Engineering

1. Motivation Current tools for the calculation of 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. The aim of this study is to develop a country-level inventory for global coal-fired power plants 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 (WEP) 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 Pollution 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

C. Oberschelp, ETH Zurich; S. Pfister, S. Hellweg, ETH Zurich / Institute of Environmental Engineering

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) are site-specific inventory for airborne coal-fired 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 Pollution 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.

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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**
Reorganisation of a long-term monitoring network using mosa as bio-monitor for atmospheric deposition by example of Germany
S. Nickel, University of Vechta / Chair of Landscape Ecology; W. Schröder, University of Vechta / 2
The determination of atmospheric deposition can be accomplished using technical sampling devices (bulk samplers, wet only samplers), biomonitoring or modelling. In Europe, since 1990 moss sampled every five years at up to 7000 places in up to 35 countries was used as biomonitor. In the moss species, 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 reorganised. In this context, a problem with the biomonitoring is the environmental quality of the network, 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 reorganised network did not reveal significant loss of statistical validity.

**WEPC15**
Spatial patterns and temporal trends in contamination of Norwegian coastal biota
A. Poste, Norwegian Institute for Water Research; G. Everaert, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; D. Hjermann, Norwegian Institute for Water Research (NIVA); D. Green, University of Saskatchewan - Toxicology Centre / Toxicology; K. Borgia, Department of Biosciences, University of Oslo / Department of Biosciences; A. Ruus, NIVA / NIVA
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 of 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 Baltic Sea
Y. Deschutter, Ghent University (UGent); G. Everaert, Ghent University / Laboratory of Environmental Toxicology and Aquatic Ecology; K. De Schamphelaere, Ghent University (UGent) / Applied Ecology and Environmental Biology; M. De Troch, Ghent University / Marine Biology
Marine ecosystems are increasingly experiencing multiple disturbances as climatic change leads to ongoing changes in sea water temperature, salinity, and pH. When increased nutrient concentration and pollution 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 interact in 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 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 stressors. By means of statistical methods, this study demonstrates that the major stressors influencing the abundance, composition and distribution of marine zooplankton in the BPNs, and their interactions, can be comprehensively described and modeled, thus providing an improved understanding of mechanisms controlling the distribution of these key functional groups in the Baltic Sea. This understanding is crucial for predicting the effects of climate change on marine ecosystems.

**WEPC17**
Data science to link chemical contamination with biological effects in surface water
M. Van der Donck, ARCHE; L. Audoom, Boobook
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 PAEs), biological parameters (species counts/indices for macro-invertebrates, fish, macrophytes and phytoenbenthos) 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
D. De Zwart, DZL Ecosys / Centre for Sustainability Environment and Health; L. Posthuma, RIVM / Centre for Sustainability, Environment and Health
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 EKFs. In a series of five reports and two computer application tools the methodology to assess the EKF-toxicity is described along two different 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 biosassays. 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 retrieved from a variety of sources. This exercise yielded a total of 94720 toxicity records, comprising 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 exchange and quality of the SSD dataset is categorized. The computer application to derive overall mixture toxic pressure expressed as the multi-substance Potentially Affected Species of mixPAF 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.

**WEPC20**
Environmental endocrine compound concentrations and human and ecosystem health effects (PC)
Effects on thyroid signaling and early neurogenesis of pesticides amitrole and chlorpyrifos

P. Spirihnlova, MNHN / UMR721; D. Du Pasquier, Watchfrog S.A.; S. Le Mevel, MNHN / UMR721; C. Bornheg, Karlstads universitet; B. Mughal, MNHN / UMR721; C. Lindb, B. Jonsson, Larsun universitet; J. Fini, MNHN / UMR721; B. Demeneix, MNHN

Abstract: During development, the thyroid gland has been demonstrated in several species. Our goal was to use transgenic TgHβZ-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 long-term 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 microarray and RT-PCR methodologies. We found upregulated proliferation activity at concentrations 10^{-4} M (amitrole) and 10^{-6} M (chlorpyrifos). In addition, 10^{-4} M amitrole exhibited anti-thyroidogenic activity in the presence of T3 (5nM). Amitrol (10^{-3} M) and chlorpyrifos (10^{-3} M) significantly reduced the mobility of tadpoles. Both compounds significantly reduced the expression of mature neuronal marker tubb2b at total concentration 10^{-3} M. Chlorpyrifos (10^{-3} M) also reduced the expression of cntn6 (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.

WECPC2
Urinary concentrations of major phthalate metabolites in pregnant mothers and newborns in relation to thyroid hormones

S. Kim, Seoul National University / Department of Environmental Health; K. Choi, Seoul National University / Environmental Health Sciences; S. Kim, Seoul National University, Graduate School of Public Health / Department of Environmental Health; H. Moon, Hanyang University / Marine Sciences and Convergent Technology; J. Park, SOON CHUN HYANG UNIVERSITY / College of Natural Sciences

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 within 24h of delivery, 152 newborns, first urine samples were collected. Six phthalate metabolites for three phthalates, i.e., di(2-ethylhexyl) phthalate (DEHP), di-isobutyl phthalate (DBP), 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 MIBP levels and 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. Implications of thyroid hormonal change to fetus are of concern, and thus deserves further consideration.

WECPC3
Mixtures affecting neurodevelopment disrupt thyroid hormone signaling in Xenopus laevis

M. Borheg, MNHN / UMR721; P. Spirihnlova, S. Le Mevel, MNHN / UMR721; C. Bornheg, Karlstads universitet; B. Mughal, MNHN / UMR721; C. Lindb, B. Jonsson, Larsun universitet; J. Fini, MNHN / UMR721; B. Demeneix, MNHN

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 (SEMLA 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. THyroid hormones (THs) are crucial for brain development. We hypothesized that this axis could be a key target for disruption and hence alter neurodevelopment. In order to evaluate this hypothesis, we used 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 TgHβZ-GFP 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 1x and 1000x concentrations. In co-treatment with T3 (5nM), Mix N exhibited anti-thyroidogenic activity at 10x 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 in 1000x on the transcription of thyroglobulin and transcription factor kbif in presence of T3. Without T3, exposure to Mix N 10x downregulated expression of outl1c1, 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 experimental mixtures can be used to unravel the mixtures associated with adverse outcomes in humans is promising. It offers a connection between adverse effects in human, chemicals and mechanistic data promoting better mixture risk management.
example fish, might become observable in a later stage of development, during reproduction, or even in the next generation. Moreover, thresholds 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 (14C-EE2) for a short time under exactly the same conditions via two different routes (via the water: 1 μg 14C-EE2/L and via the diet: 0.27 μg 14C-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**

C. Carmean, Kobe University / Department of Physiology and Cell Biology; A.G. Kirkley, University of Chicago / Division of Biological Sciences; N. Yokoi, Kobe University Graduate School of Medicine / Department of Physiology and Cell Biology; R. Hoshikawa, Kobe University / Department of Physiology and Cell Biology; R.M. Sargis, University of Chicago / Section of Endocrinology, Diabetes, and Metabolism; S. Seino, Kobe University / Department of Physiology and Cell Biology

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.
Glucose-stimulated insulin secretion. Unexpectedly, glucose-stimulated calcium metabolic dysfunction. We system by which we can interrogate the underlying mechanisms of arsenic-induced 
vitro interrogated this possibility using a model of chronic low-dose arsenic exposure in glucose tolerance test, plasma glucose was significantly elevated at all time points effects on diabetes, animal and cell-culture models suggest that arsenic exposure

Biology and Metabolism; S. Seino, Kobe University / Department of Physiology and Cell Biology; R.M. Sargis, University of Chicago / Section of Endocrinology, Diabetes, Chronic Arsenic Exposure Impairs Pancreatic

Extended One Generation Reproduction Test (MEOGRT, OECD 240) and the prolong test scenarios (with a flow through design), as for example the Medaka

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and age, which are difficult to

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Keyword Index

Biodegradation.

Aquatic toxicity.

Bioavailability.

Bioaccumulation.

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Keywords.

Acute toxicity.

Adsorption.

Ammonia.

Acidity.

Acidosis.

Acclimatization.

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Acclimation.
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Mixture toxicity.

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Pesticide.

Risk assessment.

Nanomaterials.

Nutrients.

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Mixture toxicity.

Microcosm.

Mixture toxicity.

Mixture toxicity.

Mixture toxicity.

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