NORTHTOX 2023
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Abstracts for oral presentations in the sessions

1

Mind the gap: Cosmetic preservatives and their regulation

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Abstract

Chemical regulation is a key component in the transition towards a sustainable and toxic-free society. Current EU chemicals legislation is complex and consists of more than fifty regulations and directives, each with its aim and scope. In practice, one chemical may fall under several different regulations, depending on its use. This may lead to inconsistencies in regulatory obligations, thus compromising the high level of protection of human health and the environment.

One such example is antimicrobial substances used as preservatives in cosmetic products. Due to their ability to affect living organisms, cosmetic preservatives are subjected to risk assessment under the Cosmetic Products Regulation. However, the risk assessment only considers the risks to human health and not to the environment. Environmental hazards of cosmetic substances should instead be managed by the REACH Regulation. The same antimicrobial substances may also be used as biocidal substances in biocidal products, regulated by the Biocidal Products Regulation. This use requires risk assessment and authorization of both the antimicrobial substance and the final biocidal product.

We conducted a qualitative document analysis of the Cosmetic Products Regulation and Biocidal Products Regulation to determine differences in the regulation of antimicrobial substances. In addition, we used a case-study methodology to investigate how the REACH Regulation can manage environmentally hazardous cosmetic preservatives. We found the regulation of antimicrobial substances to be stricter when used in biocidal products. We also found that environmentally hazardous cosmetic preservatives were not likely to be effectively managed by the REACH Regulation.

2

Technology-critical elements - assessment of the risks

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Abstract

Technology-critical elements (TCEs) include 27 specific elements (14 out of the 17 rare earth elements, the 6 platinum group metals and another 7 elements; Ga, Ge, In, Nb, Ta, Te, Tl) in the periodic table and are characterised by 1) their useful properties and enormous use in a wide range of new (green) technological applications, and 2) their scarcity relative to the enormous demand. Alarmingly, the widespread use of these elements has led to their presence in elevated concentrations in several environmental compartments; soil, ground- and surface water, sediments, glaciers, etc. At the same time, we know very little about their properties and behavior in the environment.

With the overall aim of protecting human health, the TCEs are risk assessed in three sub parts in this VR-funded Ph.D. project. In the first, uptake of TCEs from soil into (edible) vegetables is evaluated, both under current conditions and in a simulated and plausible future scenario with higher soil concentrations. In the second part, potential toxicity is investigated with in vitro bioassays. The third part aims to assess human bioaccessibility after oral exposure and will be performed using the so called BARGE Unified Bioaccessibility Method (BARGE-UBM).

The oral presentation mainly covers part one, but to get an overall perspective, a shorter overview of parts two and three (not yet started in practice) is given as well.

3

Comparing sensitivity of pubertal and adult 28-day exposure scenarios in female rat toxicity studies using diethylstilbestrol or ketoconazole

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Abstract

Exposure to environmental chemicals may have negative effects on female reproductive development and, ultimately, women's fertility. However, current test methods seem inadequate in detecting female reproductive toxicants, which relates to lack of knowledge on optimal timing of exposure, sample collection and sensitivity of assessed endpoints. In this study we used two well-known human endocrine disrupting chemicals, diethylstilbestrol (DES; 0.003, 0.012, 0.048 mg/kg bw/day, strong estrogen) and ketoconazole (KTZ; 3, 12, 48 mg/kg bw/day, affects steroidogenesis), to investigate if time of exposure affected endpoint sensitivity. Using a 28-day in vivo toxicity study, we exposed rats via oral gavage in a pubertal (postnatal day (PND) 22~50) or an adult (PND 64~90) exposure regime. The same endpoints were investigated for both exposure periods; estrous cyclicity, organ weight and follicle composition. For the pubertal study, day of vaginal opening (VO) was also included. We found that DES exposure altered estrous cyclicity, reduced ovary weight, as well as number of antral follicles and corpora lutea in both exposure regimes, and an advanced day of VO was observed. KTZ increased ovary weight and number of antral follicles after adult exposure, but not after pubertal exposure. Adrenal weight was increased after both pubertal and adult exposure to KTZ. As the differences between the two exposure scenarios were not very pronounced, a clear preference for one exposure setting over the other cannot be determined.

4

Image-based High-Content Screening of Mitochondrial Membrane Potential in Daphnia magna

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Abstract

The high complexity of interactions between biota and anthropogenic chemicals requires methods that are able to rapidly provide information about sublethal effects and toxicological mechanisms. We are using a novel approach based on molecular stains and high-content screening (HCS) to predict toxicological effects and provide valuable information about a chemical's mode of action in the ecotoxicological model organisms Daphnia magna . This study focused on the applicability of the JC-1 stain that permeates both the cell and mitochondrial membrane. JC-1 forms red-orange light emitting J-aggregates when the mitochondrial membrane potential (MMP) is high, and stays in its green-light emitting monomeric form when the MMP is low. The red to green signal ratio is therefore both a measure of cell viability and an indicator of MMP interference. First experimental workflows for HCS in D.Magna were developed. We then exposed D.Magna juvenilies to a concentration series of 2,4-Dinitrophenol (2,4-DNP), which interferes with the MMP by uncoupling the oxidative phosphorylation. After 2 h and 24 h of exposure, the juveniles were stained with JC-1 in microwell plates and analyzed with an ImageXpress Micro confocal microscope, using automated image analysis and data evaluation in R.

5

Retinoic acid disrupting chemicals perturb germ cell development in the fetal mouse testis: causal evidence for Adverse Outcome Pathway no. 400

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Abstract

Timely and accurate retinoic acid (RA) signaling is pivotal for reproductive health by regulation of meiosis. In the fetal ovary, the oogonia enter meiosis under the influence of RA whereas the prospermatogonia avoid entering meiosis prematurely by active removal of RA from the fetal testis. Several environmental chemicals can interfere with RA signaling. We hypothesize that disruption of RA degradation in the fetal testis can lead to ectopic RA expression and hence premature meiotic initiation with consequences for adult fertility. We are currently building an Adverse Outcome Pathway (AOP400) describing these causal events. Existing evidence is primarily derived from developmental biology studies, with limited knowledge available for chemical toxicity. To provide additional toxicological data, we have examined effects of chemical disruption of RA signaling during fetal testis development. Many azole fungicides can interfere with RA signaling. Using an ex vivo culturing system and transgenic mouse lines we investigated the effects of a panel of azoles on RA signaling during gonadal sex differentiation. Fetal mouse testes were collected on gestational day 13.5 and cultured in hanging drops for 48 hours exposed to either vehicle, RA, triticonazole, flusilazole, ketoconazole, triadimenol or prochloraz.

Azole exposure induced ectopic RA response element (RARE) activation and RA signaling in ex vivo cultured fetal mouse testes. This affected germ cell development by inducing premature meiotic entry, indicated by induction of meiotic marker STRA8.

Our study suggests that azoles can induce ectopic meiosis in prospermatogonia via perturbation of the retinoid signaling pathway.

This is strong evidence supporting AOP400.

6

Male-transmitted transgenerational effects of the herbicide linuron on the DNA methylome in testis and brain of Xenopus tropicalis frogs

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Abstract

Unsustainable human activities are driving the ongoing biodiversity loss, and amphibians constitute a particularly threatened group. Our recent study showed that the herbicide linuron can cause endocrine disrupting effects in Xenopus tropicalis frogs, including subsequent generations that were never exposed to the contaminant. However, the mechanisms by which these effects are transmitted across generations need to be further investigated. In this study, we examined the transgenerational effects of the herbicide linuron on DNA methylation patterns in the brain and testis of X. tropicalis . Tadpoles were exposed to an environmentally relevant concentration of linuron during development, and adult males were mated with naïve females to obtain the F1 and F2 generations and follow paternally transmitted effects. Reduced representation bisulfite sequencing (RRBS) was used to assess DNA methylation patterns in the brain and testis of the adult male F2 generation. Differentially methylated regions (DMRs) were identified in genes related to growth, metabolism, and germ cell development. The DMRs also included genes that are vital for regulating the epigenetic landscape. These findings are in accordance with previously shown phenotypical alterations and suggest that developmental exposure to linuron can cause transgenerational alterations in DNA methylation patterns that affect growth, metabolism, reproductive function and epigenetic regulation in amphibians. This study contributes to the understanding of the potential mechanisms of transgenerational effects of pollution in amphibians and emphasizes the need for further research to elucidate the long-term effects of environmental contaminants on wildlife populations.

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The GARDskin assay adopted into OECD TG 442E – The first and only genomics and machine learning based in vitro skin sensitization assay – Assay overview and industry-based case studies

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Abstract

Hypersensitivity reactions in the skin, clinically manifested as Allergic Contact Dermatitis (ACD), are caused by the ensuing immunological response to low-molecular weight compounds termed skin sensitizers. Traditionally, testing has been performed using a combination of in vitro and in vivo assays, but recent developments has shifted the paradigm towards the use of New Approach Methodologies (NAMs), without the need for in vivo methods. Several NAMs has to date been validated and adopted as OECD test guidelines, each one covering key events of the adverse outcome pathway for skin sensitization. However, lack of broad applicability domain of the existing guidelines opens for new assay to fill data gaps where current assays are not applicable.

The GARD®skin assay was recently adopted into OECD TG 442E. As the first test guideline combining genomics and machine learning for hazard identification of sensitizers, it extends the applicability domain of NAM-based assays by documented capacity to accurately predict challenging substances such as mixtures, indirectly acting haptens, lipophilic compounds and metals.

Here, the GARDskin assay and its applicability is presented in the form of several case studies taken from industry collaborations on difficult-to-test substances.

In conclusion, this compilation of case-studies demonstrates the applicability of the GARDskin assay and how the data generated can be used for regulatory and R&D purposes.

8

High-content analysis shows tumorigenic activity of per- and polyfluoroalkyl substances (PFAS) as single compounds and mixtures in human breast epithelial cells

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Abstract

Perfluoroalkyl substances (PFAS) have been associated with cancer, but the potential underlying mechanisms need to be further elucidated and include studies of PFAS mixtures. Although the production of PFOS and PFOA has been phased out, they are still found in the environment and humans,and the shorter-chain replacement PFAS are increasingly detected.

Here, we applied high-content image analysis (HCA) to investigate the effects of 6 short-chain PFAS and a mixture of PFOS and PFOA on proliferation and transformation of normal human breast epithelial cells (MCF-10A). The results revealed that PFHxA, GenX, PFO2OA, HFBA and PFBS induced no alteration compared to controls, while PFHxS at 100 µM induced cell proliferation. Interestingly, very low concentrations (500 pM) of the binary PFOS and PFOA mixture induced synergistic effect on MCF-10A proliferation. PFHxS and the PFAS mixture also altered histone modifications and promoted cell migration and invasion by reducing levels of adhesion proteins. The HCA assay cell painting, which includes multiplexing of six stains and automatic image analysis, was used to capture large amounts of unbiased information to quantify the cellular state after exposure. Hundreds of cell features were affected by the PFAS mixture even at the lowest concentration tested (100 pM).

Increased knowledge about mixture-induced effects is important for better understanding of PFAS' possible role in cancer etiology, and may impact the risk assessment of these and other compounds. This study further shows the potential of image-based multiplexed fluorescence assays and high-content screening for development of new approach methodologies in toxicology.

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Enzymatic methyl whole genome sequencing shows perfluorooctanesulfonic acid induced cancer related methylome alterations in human breast cells

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Abstract

Environmental contaminants can influence epigenetic mechanisms that regulate the interactions between genetic and environmental risks factors during carcinogenesis. Perfluorooctanesulfonic acid (PFOS) has been associated with breast cancer, but the information about its effect on DNA methylation is sparse.

Here we exposed human normal breast epithelial (MCF-10A) cells to PFOS and examined alterations in the DNA methylation landscape using enzymatic methyl whole genome sequencing (EM-seq). Differential methylation was investigated at base pair resolution (CpG-sites) and regions of 100 base pairs, by applying logistic regression. In total, we identified 16’075 differentially methylated CpG-sites (DMCs), which overlapped with 8’057 genes and 485 CpGislands (CGIs). We also identified 1'116 differentially methylated regions (DMRs), which overlapped with 776 genes and 24 CGIs. Several of these genes have been shown to be involved in cancer development and prognosis. Functional enrichment analysis revealed gene ontologies related to (1) neural development, (2) cell and membrane adhesion, (3) actin activity and (4) ion channel activity, with the top ten genes being: PTK2B, CDH2, ACTN2, RHOA, GRIN2A, BAIAP2, CTNNB1, CACNA1C, GRIN2B and GRIN1.

To our knowledge this is the first whole-genome methylome study to investigate the effects of PFOS at single CpG-site resolution. The findings contribute to identify specific genes that are differentially methylated by PFOS, and improves the understanding of its potential role in breast cancer etiology. In a wider context, it illustrates the potential of NAMs and new epigenomics methods to elucidate mechanisms underlying adverse effects of chemicals, generate candidates for biomarkers, and strengthen epidemiologic associations.

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Morphological profiling as an emerging tool in toxicology of particles and chemicals

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Abstract

The next-generation toxicology tools should imply the use of nontargeted, high-throughput profiling assays for initial characterization of the biological activity of toxicants, e.g., PAHs, PFAS, particulates, plastics-associated chemicals, etc. Cell Painting is high-throughput and high-content morphological profiling assay that reveals impact of perturbation(s) on eight broadly relevant cell compartments, including nucleus, actin/Golgi/plasma membrane, mitochondria, endoplasmic reticulum, and RNA/nucleoli. In this study, we have exposed a number of human cell lines (U-2 OS, HepG2, HepaRG, A549, THP-1) to various toxicants including a panel of PAHs, PFAS mixtures, plastics-extracted chemicals, prototypical nanoparticle models, unintentionally generated (nano)particle emissions at the occupational setting, and known activators of NLRP3 inflammasome. Afterwards, cells were processed by the Cell Painting protocol and image analysis was performed by using CellProfiler software. CellProfiler measured ~3.200 morphological features of single cells related to size, granularity, shape, texture, intensity, radial distribution etc. Overall, broad panel of toxicants and a number of in vitro models enabled us to 1) identify toxicant-specific morphological profiles, 2) detect the early changes in the cell morphology at the low concentrations of various toxicants, 3) detect cell stress conditions before it can be observed in the reduced cell viability, and 4) suggest the prevailing mechanism of toxicity. Use of morphological profiling, such as Cell Painting, to study structurespecific profiles at single-cell resolution, supported with e.g., targeted assays, omics, and machine learning tools, offers great opportunities as an emerging tool in the field of particle and chemical toxicology.

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Chemical proteomics, from finding molecular targets to predicting the impact of chemicals on human health.

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Abstract

The vision of our lab is to improve our understanding of the impact of chemicals on human and environmental health. The main gaps are that the assessment of chemicals is restricted to well-characterized compounds; the exposure analysis is determined by previous knowledge of toxicity pathways; and the combined effects of chemicals cannot be estimated from uncharacterized mixtures. Our approach can circumvent those gaps by focusing on the identification of the interactions between the chemicals and the proteins in cells. We have implemented the Proteome Integral Solubility Alteration Assay (PISA) which was initially developed to identify drug targets for its application to environmental chemicals and complex mixtures. We can offer innovative methods and pipelines for high-throughput identification of protein targets from any chemical that is the first step to evaluate and predict any adverse biological effects. Our pipelines are combined with multicriteria decision-making analysis for developing Adverse Outcome Pathways (AOP) that could contribute to researchers and regulators gaining time and resources in chemicals assessment. The direct impact of this research is generating new knowledge and new resources to take early warning decisions to minimize the effects of exposure on human health. In the longer term can drive the development of new approaches for the biological validation of the possible consequences of the protein-chemical binding and rise the existing knowledge that can be coupled with the AOP framework.

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Abstract for poster presentations

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Understanding the health effects of micro- and nanoparticles collected from a road tunnel and subway station in Stockholm: Evaluation of cytotoxic, genotoxic, and inflammatory response in lung and macrophage models in vitro

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Abstract

Atmospheric pollution by particulate matter (PM) has been associated with a wide range of adverse effects. However, it is still unclear to what extent particles from various transport modes differ in toxicity and health effects. This is explored in an ongoing EUproject called nPETS (nanoparticle emissions from the transport sector: health and policy impacts) that focuses on nanoparticles (<100 nm). The main aim of this study was to compare the toxicity of micro- and nanoparticles from a road tunnel and subway station in Stockholm, with a focus on their genotoxic and inflammatory responses. This was analyzed following exposure of cultured human lung cells (A549) and differentiated macrophages (dTHP-1) by using the comet assay and multiplexing of four cytokines (IL1-β, TNF-α, IL-6, IL-8). The results showed no or only minor cytotoxicity of the particles. Cytokine release was mainly observed following exposure of the macrophages and the micron-sized particles showed similar or slightly higher effect compared to the nanoparticles. All particles tested caused an increase in DNA damage in at least one of the cell types and the nano-sized particles from the road tunnel and the micron-sized subway particles were most potent in the macrophages. Taken together, this study shows inflammatory and DNA damaging potential of both micro- and nanoparticles collected in a road tunnel and subway station in Stockholm. Studies are now ongoing to explore effects of particles in these environments on cells cultured in the air-liquid interface.

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Acute and chronic toxicity of conventional microplastic mixtures in adult and juvenile Nile tilapia (Oreochromis niloticus)

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Abstract

This project investigates the acute and chronic toxicity of conventional microplastic mixtures in adult and juvenile life stages of Nile tilapia (Oreochromis niloticus). We used the microplastic particles in a relevant size range and environmentally relevant concentration for the ingestion by fish, and non-polymer natural kaolin particles. The potential hazards of a 2 % (w/w) microplastics mixture of the conventional polymers nylon-6, polypropylene (PP), polyethylene terephthalate (PET), and high-density polyethylene (HDPE) in a size range of 10 - 350 µm were studied on adult Nile tilapia over an acute food exposure of 7 days and on juvenile Nile tilapia over a chronic food exposure of 30 days, the latter in presence of a natural particle (kaolin) control. Endpoints addressed life cycle parameters, behavior, and biomarkers for oxidative stress, inflammation, apoptosis, and intestinal health and integrity.

In both acute and chronic exposure feeding experiments with adult and juvenile Tilapia, no detectable effects of the ingestion of 2 % (w/w) MP mixture of four conventional polymers (Nylon-6, PP, PET, HDPE) were found on growth. The expression level of genes relevant to oxidative stress, apoptosis, and inflammation, biomarker assays for oxidative stress enzyme levels, and intestinal health and integrity will be analyzed in the next steps, as well as the potential accumulation of microplastics in the fish muscle after the acute and chronic exposure to the microplastics mixtures.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 860720 (LimnoPlast ITN).

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Exposure to dibutyl phthalate induces oxidative stress in vitro and in vivo

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Abstract

Phthalates are a group of chemicals that are used in many types of products, mainly in plastics. Di-butyl phthalate (DBP) is one of the most commonly used phthalates and it is considered to be an endocrine disruptor with mainly anti-androgenic effects. In the body, DBP is rapidly metabolized to mono-butyl phthalate (MBP), which is suggested to be the main metabolite causing effects in vivo . Research indicates that DBP exposure induces oxidative stress in several tissues and cell types, but studies with human model systems are scarce.

In this study, the human adrenal cell line H295R was exposed to DBP and MBP to study oxidative stress in human endocrine cells. After 2h exposure, the superoxide levels were quantified by fluorescent staining. The results showed that DBP, but not MBP, induced increased levels of superoxide in the cells. The levels of nitrotyrosine, a marker of protein oxidation, were measured after 48h exposure. Interestingly, MBP induced significantly decreased nitrotyrosine levels, whereas DBP caused no significant effect.

These results were further compared with data from our in vivo study, where adult male mice were orally exposed to 10 or 100 mg/kg/day DBP for five weeks. In the mice, a dose-dependent increase in nitrotyrosine levels was observed in both the testicles and spleen. In the spleen, the levels of other proteins involved in oxidative stress were also increased.

Taken together, these results support that DBP can induce oxidative stress in several proposed target tissues, both in mice and in a human cell model.

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A mobile ALI exposure system for toxicity testing of transportation emissions at realworld sites

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Abstract

The toxicity of airborne particles can be examined with an Air Liquid Interface (ALI) exposure system comprising cultured human lung cells. Our aim was to develop a mobile ALI system for outdoor usage, to examine toxicity of real-world particles, e.g. emissions from cars, trains, aeroplanes and ships. Advantages of our ALI system:

1. It can test the toxicity of airborne pollutants directly from a source. Particles that deposit onto cells are unchanged and match those that humans inhale.
2. It is designed to facilitate the deposition of nanoparticles, which are otherwise hard to deposit.
3) It contains separate cell chambers to avoid cross-contamination between chambers, and enable different exposure scenarios to be examined at the same time.
4) It has controlled temperature and humidity to resemble the condition in a human lung, and to keep cells alive at non-exposure conditions.
5) Deposited dose can be determined online by measuring particle size distribution before and after the ALI system.
6) Our mobile ALI system makes it possible to perform exposure measurements at out-of-the-lab locations, e.g., in road tunnels, which has not been possible before. This new design opens up the possibility to examine the toxicity of a great variety of aerosols/emissions. We expect mobile ALI systems to become a widely used tool for future assessments of aerosol´s effects on human health.

This research was supported by the European Commission's Horizon 2020 research and innovation programme: nPETS (grant agreement 954377) aimed at studying the sub-100nm particles emitted from transport.

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Toxicity of particle emissions from car and train brake materials

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Abstract

Road traffic is one of the main emitters of air pollution in cities. An important part of these emissions comes from non-exhaust sources like brakes, tires, road wear, and road dust resuspension. The characteristics of brake particles depend on the speed, load, temperature, and friction pair materials and the metallic components may cause their toxic effects. This work aimed to evaluate the toxicity (in vitro) of fresh airborne particles from 4 car brake materials and 2 train brake materials using an alveolar cell line (A549) exposed at the air-liquid interface (ALI). The particle generator (Tribometer: contact pressure 0.9 MPa, velocity 4 m/s) was connected to the ALI exposure system. Cells were exposed to airborne particles for 2h, and were then incubated for 24h before measuring the cell viability. Brake particles decreased cell viability more than the gaseous emissions (control) in three of the car brakes (FM2, FM3, and FM4) and one of the rail brakes (C6). The brakes have different metal compositions: FM1 and FM2 have a low metal content and are Cu-free, FM3 is Cu-enriched, and FM4 is nonasbestos organic (NAO) and Cu-free. In the train brakes, there is mainly Ca in C6, and Fe in C20. Moreover, the estimated exposure dose of the cells was around 4-6 µg/cm2. Further analyses will be done to better understand the toxicity of these materials by testing different particle fractions using submerged exposures. This work was supported by the European Commission's Horizon 2020 research and innovation program nPETS (grant agreement 954377).

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Cobalt skin absorption in different skin models

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Abstract

Introduction. Cobalt (Co) is a sensitizing metal able to induce skin allergy from contact. To study skin absorption, pig skin is often used. Reconstructed Human Epidermis, RhE, models could be an alternative to using animal skin.

Aim. Compare the absorption and skin retention of cobalt in the form of dissolved ions ($CoCl_2$) and nanoparticles (CoNP) using piglet skin and a RhE model.

Material and methods. Co absorption in piglet skin was studied following the OECD guideline no 428. EpiSkin™ RhE models were exposed following the protocol given by the company. Models were exposed to 10 and 100µg Co/cm2 for 4h and 24h. Phosphatebuffered saline solution was used as receptor fluid. After exposure, samples from donor, skin, and receptor were collected. Co was analysed by inductively coupled plasma mass spectrometry.

Result. Higher amounts of Co ($p=0.0286$) from CoNPs were measured in the skin compared to $CoCl_2$. A non-significant difference was observed for Co in the receptor at 24h, suggesting that the RhE model is more permeable for $CoCl_2$ (1.53µg) than for CoNPs (0.01µg). Generally, Co absorption increased with time, however, no such time dependency was observed for the Co amounts retained in skin compartment.

Conclusion. $CoCl_2$ and CoNPs were found to have different absorption profiles, which could be indicative of different routes for absorption or different release factors for ionic and particulate Co respectively. Results show similar absorption profiles of $CoCl_2$ in both models but deviates for CoNPs, indicating the importance of hair follicles in the transport of nanoparticles.

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Sociodemographic determinants associated with toxic substances in Swedish adolescents

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Abstract

Background and aim:

Few population-based studies of adolescents have investigated the relation between socio-demographic factors and the wider chemical exposome. We aim to identify associations of social demographic factors with the chemical exposome of Swedish adolescents.

Methods:

Using the Riksmaten Adolescents 2016-17 cohort (RMU) we analysed several social-demographic factors: gender, participant/maternal (P/M) birth country income level, parental education levels, and geographic location (longitude/latitude), and their relation to blood/serum/urine concentrations of a large range of toxic substances (N=64) across multiple chemical groups (heavy metals, chlorinated pesticides, PCBs, BFRs, PFASs, phthalates, phenols, pesticides, PAHs). Association analysis was conducted using ordinal regression.

Results:

P/M birth country was the most frequent determinant of toxic substance concentrations, being significantly associated with concentrations of 45 substances, followed by gender (N=41), and longitude (n=37). P/M birth country also showed the largest folddifferences in concentrations, with subjects born in high-income countries having several-fold higher estimated adjusted mean (EAM) concentrations of PCBs, BFRs and PFASs than those born in lowincome countries. In contrast, adolescents born in low-income countries had higher EAM levels of heavy metals, chlorinated pesticides, and phthalates. Substances belonging to the same chemical group tended to have the same determinants. Interesting north to south or west to east gradients of substance concentrations were identified in Sweden, suggesting geographical inequalities of exposure.

Conclusions:

P/M birth country is an important confounder and should be included in future toxicology population-based studies. Exposure associations with latitude/longitude should be further investigated.

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A cell-based platform to screen chemical mixtures for endocrine disruptive effects

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Abstract

Chemical contaminants are omnipresent in the environment and frequently detected in human samples. However, there can be great interindividual differences in exposures, and it is therefore difficult to construct a universally representative contaminant mixture. Several ubiquitous chemicals have been identified as endocrine disruptive compounds (EDCs). As the endocrine signaling systems are highly sensitive, even limited exposure to an EDC could cause adverse health effects. Current risk assessments are based on observations in single-chemical exposure studies, which is not reflective of real-life scenarios. Additive or synergistic effects are a concern, since unexpected interactions could produce an exaggerated toxicological response. The single compound approach may therefore underestimate the toxicological impact of mixture effects.

We have established a chemical test set of 26 persistent organic pollutants identified in human serum. Non-contact liquid handling dispensing is used to reproduce real-world mixtures as detected in human samples for toxicity screening. The endocrine disruptive potential of these mixtures is then investigated by using OECDvalidated in vitro cell-based methods that assess effects on steroidogenesis and interaction with androgen- and estrogen receptors.

Our aim is to establish medium or high throughput micro plate-based screening methods for toxicological investigation of single compounds and complex chemical mixtures. This workflow will later be applied to investigate the effects of reconstructed individual chemical exposomes, based on chemical profiles detected by advanced mass spectrometry analysis of human samples. This will aid in the development of relevant risk assessments for chemical mixtures, which better can protect the general population from endocrine disruptive mixture toxicity.

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Dermal bioaccessibility of residual- and listed PFAS ingredients in cosmetic products

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Abstract

Per- and polyfluoroalkyl substances (PFAS) represent a large class of synthetic chemicals which are widely used in consumer products, including cosmetics. Little is known about the importance of dermal uptake as a human exposure pathway for PFAS. In this study, artificial sweat was used to assess dermal bioaccessibility of both residual- and listed PFAS ingredients from six cosmetic products. Due to the diversity of PFAS, cosmetics and artificial sweat extracts were first screened for the presence of fluorine by combustion ion chromatography (CIC), and thereafter for individual PFAS by gas- and liquid chromatography- mass spectrometry. Among residual PFCAs, bioaccessibility generally increased with increasing chain length (54% for PFBA to 95% for PFOA). Concentrations of individual polyfluoroalkyl phosphate esters (PAPs), which were listed ingredients in one product, were below detection limit in sweat, indicating low bioaccessibility (<0.002%). This work provides the first estimate of dermal bioaccessibility for both residual- and listed PFAS ingredients in cosmetic products and helps to further our understanding of the importance of the dermal pathway towards overall human exposure to PFAS.

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Is it possible to identify endocrine disruptors without animal tests? A case study on PFOS

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Abstract

Endocrine disruptors (EDs) are of high concern, and there is substantial work going on in the EU to identify and regulate this hazard. Currently, the process of identifying EDs in the EU require a significant amount of animal data. This goes against the aim, within both the regulatory and the scientific community, to reduce and replace animal testing of chemicals. Simultaneously, there are remarkable developments in non-animal test methods and concepts like the Adverse Outcome Pathway (AOP) framework. The AOP framework has also been highlighted as a promising tool to integrate data from novel methods and is well-suited for the ED assessment. This study investigates if perfluorooctane sulfonic acid (PFOS) fulfils the EU scientific criteria as an ED. The ED assessment was conducted according to current EU guidance for identifying EDs, and through an additional mechanism-based approach. Identification and selection of relevant studies was done in a rigorous way using Systematic Review methodology, and study reliability was evaluated using the SciRAP tool (www.scirap.org). Two separate Weight-of-Evidence (WoE) assessments were performed, once using all available data and again without in vivo animal data. Furthermore, the project implements the use of an AOP network to investigate use of AOPs in the ED identification process.

According to the WoE assessment, PFOS fulfils the scientific criteria as an ED with strong evidence linking thyroid disruption to developmental neurotoxicity. Preliminary results from the mechanism-based assessment highlight several possibilities and challenges for identifying EDs without the use of in vivo data.

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Exposure to a human-relevant mixture of endocrine disrupting chemicals induces changes in hippocampal DNA methylation correlating with hyperactive behavior in male mice

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Abstract

Humans are constantly exposed to environmental chemicals e.g. endocrine disrupting chemicals, which can result in adverse health effects. In utero exposure is considered a particularly sensitive window in which exposure can lead to long-lasting adverse effects. Epigenetic mechanisms such as DNA methylation are likely involved in such long-lasting changes. In a previous epidemiological study, in utero co-exposure to 8 EDCs was associated with delayed language development in children, suggesting an impact on neurodevelopment. In utero exposure to this mixture in human-relevant concentrations was shown to alter gene expression and behavior in adult mice. To address whether epigenetic alterations could underlie the observed molecular and phenotypic changes induced by the mixture, we analyzed DNA methylation at the changed genes and investigated whether it is correlated with altered gene expression and behavior. Using bisulfite-pyrosequencing, we analyzed DNA methylation at regulatory regions of these genes and identified hypomethylation in three HPA axis-related genes, namely Nr3c1, Nr3c2, and Crhr1, coding for the glucocorticoid receptor, mineralocorticoid receptor, and corticotropin releasing hormone receptor 1, respectively. Nr3c1 methylation was negatively correlated with Nr3c1 expression. Additionally, we found that the lower DNA methylation in Nr3c1, Nr3c2, and Crhr1 correlates with activity under stress conditions, and, for Crhr1, also with decreased social behavior. Our results show that in utero exposure to a human-relevant EDC mixture induces epigenetic changes directly correlated to long-lasting behavioral effects.

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From human exposure to cell cultures: a method to compare doses using a case study on welding fume nanoparticles

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Abstract

New strategies in toxicology call for approaches including in vitro to in vivo extrapolation (IVIVE). But how can doses be compared between nanoparticle concentrations in the air (µg/m3) of exposed humans and those used in cell cultures? The best option is to estimate the cell dose (µg/cm2) of deposited particles. The aim of this study was to explore the lung deposition and retention of welding fume particles and to relate the doses to our own studies on cultured cells. Data on welding fume particle concentrations and size distributions were derived from the literature and used as input to the Multiple-Path Particle Dosimetry model (MPPD). The MPPD simulated exposure scenarios for 1 week up to 45 years, assuming 5 working days/week with 6 h/day of exposure. Already after 6 h exposure (size 50 nm, concentration 5 mg/m3), the tracheobronchial lung dose (0.89 µg/cm2) exceeded the in vitro cytotoxic cell dose (0.125 µg/cm2) previously determined by us in human bronchial epithelial cells. Yet, the tracheobronchial retention dropped quickly when exposure halted, in contrary to the alveolar retention which accumulated over time and exceeded the cytotoxic in vitro cell dose after 1.5 working weeks. When simulating lifelong exposure to lowend concentration (size 50 nm, concentration 0.05 mg/m3) the alveolar retention became comparable to the cytotoxic in vitro dose after 15–20 years of welding. This study highlights the potential of using particle deposition modeling together with occupational exposure data to enhance the translation between in vitro doses and human exposure.

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The Partnership for the Assessments of Risks from Chemicals: an important EU initiative for the future regulation of chemicals in Europe

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Abstract

Society demands chemicals that are not toxic, bioaccumulative or persistent while at the same time using fewer animal experiments. To provide knowledge in support of these policies the EU has initiated the **Partnership for the Assessments of Risks from Chemicals (PARC)** as part of the Horizon Europe program. It is the first partnership of its kind to have EU agencies alongside national agencies addressing issues on the availability of chemical data and methods.

Two hundred institutes across Europe are partners in PARC. The objectives are to improve methods for exposure assessment in humans & the environment, develop New Approach Methodologies to assess the safety of chemicals and develop new and improved methods for risk assessment of chemicals for both humans & the environment.

The Norwegian Institute of Public Health is the national hub for the 8 other Norwegian institutes that are partners in PARC. The focus is on developing New Approach Methodologies to identify and characterise immunotoxic compounds and to improve a Developmental Neural Toxicity (DNT) in vitro battery using an established neural stem cell model. Separately, to increase our mechanistic understanding, Adverse Outcome Pathways (AOP) will be developed linking a molecular initiating event with key events leading to an adverse outcome. NIPH is leading working groups that will develop AOPs for immunosuppression and for DNT.

The newly generated data and strengthened research networks in PARC are of direct relevance to regulatory work on a national, European- (ECHA & EFSA) and international level (WHO & OECD).

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Evaluation of solid-phase extraction methods for effect-based assessment of hazardous chemicals in water

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Abstract

Water sample preparation for in vitro bioassays often include solid phase extraction (SPE). The pollutant burden in a water sample can range from pico- to micro molar levels depending on the source of the water. SPE is used to enrich organic micro-pollutants to increase the sensitivity of the bioassay. SPE is performed by passing a water sample over a column packed with a sorbent material that retains the pollutants as the water flows through.

As water samples can be contaminated with a complex mixture of chemical pollutants, it is desirable to use SPE sorbents that can capture as broad range of pollutants as possible. Additionally, it is vital that the sorbent material does not contaminate the sample with compounds causing activity in the bioassay(s).

Here, we have assessed three commercially available SPE columns and one custom made column for recovery of bioactive reference compounds in spiked pure water. Additionally, we have evaluated the potential contamination of water samples from sorbent materials by processing pure water controls. The extracts were assessed in a battery of commonly applied bioassays for water quality assessment. Endpoints as AhR activity, ER- and AR activation as well as oxidative stress response (Nrf2 activation) were included.

The results show differences in recovery between the columns and indicate that some sorbent materials might contaminate samples with bioactive compounds, which could interfere with the bioanalysis. It is evident that the choice of SPE column can affect the outcome of the bioassay.

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Risk-driving PFASs cocktail in Swedish human blood

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Abstract

Per- and polyfluoroalkyl substances (PFASs) are a group of anthropogenic chemicals used because of their unique combination of both hydrophobic and heat-resistant properties. Based on epidemiological studies, human biomonitoring guidance values (HBM-GVs) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) in human blood have been derived, above which a risk of adverse effects cannot be excluded. The aim of this study was to see how many individuals were above risk threshold for PFOA and PFOS in the general population of Stockholm.

In this study, 60 individuals were analysed, along with a pooled sample of 100 individuals. The samples were taken from healthy blood donors in Stockholm 2020. In short, 0.5 mL serum was extracted using 4 mL acetonitrile and ultrasonicated for 15 minutes, and repeated once. Granular ENVI-Carb (25 mg) and 50 µL glacial acetic acid was used for clean-up. The samples were analysed on a HPLC-MS/MS. Hazard Index (HI) was used for the mixture risk assessment (MRA) by adding the hazard quotient (HQ) of each chemical. The HQ was calculated by dividing the individual blood concentration of one chemical by the HBM-GV of the same chemical. All samples had quantifiable levels of both PFOA and PFOS. Thirty eight percent of the individuals were above the risk threshold for either PFOA or PFOS (HQ>1). The MRA (PFOS and PFOA) showed that the pooled sample and 75% of the individuals were above the risk threshold indicating that a risk of reproductive, developmental and immunological effects in humans cannot be excluded.

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Per- and polyfluoroalkyl substances (PFASs) in dog serum

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Abstract

Dogs share environment with their owners and are thus exposed to a similar mixture of environmental chemicals through the common indoor environment. Our aim was to determine the exposure of pet dogs to a range of per- and polyfluoroalkyl substances (PFASs).
Blood samples were collected from the cephalic vein of male dogs (n=67) and serum stored frozen awaiting analyses. We used a combined targeted and suspect screening approach that facilitates quantitative analysis of 50 PFASs, simultaneously collecting data dependent product ion scans on masses from an in-house suspect list of over 200 PFASs. Blood serum (200 - 500 µL) was extracted with acetonitrile and cleaned-up with graphitized carbon. Instrumental analysis was accomplished using an ultra-high performance liquid chromatograph coupled to a Q ExactiveOrbitrap mass spectrometer.
The relative standard deviations of the most frequently reported PFASs were 12-144%. Nineteen PFASs were above detection frequency in >30% of the samples, while six PFASs were above limit of quantification in >30% (PFBS, PFDA, PFHxS, PFNA, PFOA & PFOS). Median concentration in serum of the dominating PFASs were 1.33 ng g-1 PFOS (5th-95th percentile 0.32-5.5), 0.33 ng g-1 PFHxS (0.05-0.95) and 0.22 ng g-1 PFOA (0.03-0.79).
The concentrations and PFAS profile were similar to what is commonly reported in human serum, demonstrating that humans and pet animals are sharing PFAS exposure. Next, the suspect screening analysis will be investigated, and the PFAS results will be associated to sperm quality biomarkers.
Funding was received from Mats F. and Catharina Linde Forsbergs foundation, SLU Future One Health platform.

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Environmental chemicals change extracellular lipidome of mature human white adipocytes

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Abstract

Certain environmental chemicals affect body's energy balance and are known as metabolism disrupting chemicals (MDCs), which have been implicated in the development of metabolic diseases (obesity, type 2 diabetes). MDC effects on mature adipocytes or adult adipose tissue, are largely unclear.

We investigated the effects of six environmental MDCs (bisphenol A (BPA), perfluorooctanoic acid (PFOA), triclosan (TCS), p,pdichlorodiphenyl-dichloroethylene (ppDDE), tributyltin chloride (TBT) and triphenyl phosphate (TPP)), in 3-day-long exposure on mature human white adipocytes derived from mesenchymal stem cells. We aimed to identify sensitive endpoints of their metabolism disrupting effects. While most of the exposures had no effect on cell-based endpoints, the highest concentration of triclosan affected lipid storage, adipocyte size, glucose consumption and expression of glucose transporter GLUT1, leptin and adiponectin.

In contrast, the lipidomic analysis of the cell culture medium was extremely sensitive and revealed concentration-dependent changes in the extracellular lipidome of adipocytes exposed to nearly all studied chemicals. While some of the lipidome changes were specific for the MDC used, some effects were common to several chemicals and included increases in lysophosphatidylcholines, glycerophospholipids and ceramides and a decrease in fatty acids, with possible implications in inflammation, lipid and glucose uptake. This study points to early signs of metabolic disruption and likely systemic effects of mature adipocyte exposure to environmental chemicals, and to the need to include lipidomic endpoints in the safety assessment of MDCs.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement GOLIATH grant No.825489.

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Toxicology in the anthropocene – how do we adapt the educational curriculum for preparing students to take future lead in the transformation to a safe and sustainable society?

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Abstract

Human activity is now putting a great pressure on the natural functions of Earth and we are trespassing multiple so-called planetary boundaries. These are quantitative boundaries within which humanity can continue to thrive for generations to come. The challenges include e.g. climate change, biodiversity loss and chemical pollution. Education within toxicology, as well as other disciplines, needs to be developed to prepare students to take future lead in the work for sustainable development. Higher education is often characterized by a focus on specialization. Within toxicology it might be straightforward to teach aspects of sustainable development that relate to emerging chemicals, new methods and novel risk assessment approaches. This is critical. But education for sustainable development also calls for skills such as critical and ethical thinking and reflection, an ability to create visions and partnerships as well as self-awareness. Such aspects are preferentially trained using non-traditional pedagogical approaches including an active student-centered approach and co-creation of the content. Examples and experiences from the Global Master's Programme in Toxicology at Karolinska Institutet will be presented with an emphasis on intended learning outcomes, content and teaching approaches used in the course “Global toxicology in a sustainable society”. Challenges and future needs will be discussed with the intention to start a dialogue among teachers at different universities. The goal is an educational curriculum that promotes students to gain both an ability and a will to work for sustainable development.

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LONG-TERM EFFECTS OF PERINATAL EXPOSURE TO A GLYPHOSATE-BASED HERBICIDE ON THE MELATONIN LEVELS AND OXIDATIVE BRAIN DAMAGE IN ADULT MALE RATS

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Abstract

Concerns have been raised regarding the potential adverse health effects of the ubiquitous herbicide glyphosate. Increasing scientific evidence indicate that glyphosate-induced oxidative stress is the main toxicity mechanisms responsible for cell damage. Here, we investigated long-term effects of developmental exposure to a glyphosate-based herbicide (GBH) by analyzing serum melatonin levels and cellular changes in the striatum. Pregnant and lactating rats were exposed to 3% GBH (0.36% glyphosate) through drinking water from gestational day 5 to postnatal day 15. The male offspring showed reduced serum melatonin levels at adult age (90-days old) compared with the control group. Mechanistic in vitro studies with primary rat pinealocytes exposed to 50 µM glyphosate demonstrated a decreased melatonin secretion partially through activation of metabotropic glutamate receptor 3 (mGluR3), while higher glyphosate levels (100 or 500 µM) also reduced the pinealocyte cell viability. The perinatal exposure to GBH also impaired the redox balance of the adult striatum demonstrated by increased lipid peroxidation and DNA/RNA oxidation together with increased levels of superoxide dismutase (SOD1). Moreover, GBH treatment significantly increased the number of tyrosine hydroxylase (TH)-positive neurons in the striatum. The GBH-mediated oxidative stress caused by the imbalance between ROS production and the antioxidant defense system together with the persistent neuroendocrine deficits reported here may potentially be involved in the etiology of several chronic and neurodegenerative diseases.

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Application of NAMs in the reproductive toxicity hazard assessment and mechanistic analysis within the REACH and CLP regulation, a case study.

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Abstract

Historically, animal testing has been used for reproductive toxicity assessment under REACH/CLP regulation. In this project a new in vitro- screening assay (ReproTracker®) and a systematic literature search was used in the reproductive toxicity assessment and mechanistic analysis of two industrial compounds. The aim of this project was to investigate how NAMs could be utilized in reproductive toxicity assessment under REACH/CLP.

The existing toxicological studies under REACH was scrutinized and the reproductive toxicity classification criteria under CLP was evaluated, using the compounds as examples. The methods applied was a systematic literature search and a WoE assessment. Both compounds was also screened for embryotoxicity in the ReproTracker® and the applicability of the model was evaluated. The result from the ReproTracker® identified both compounds as embryotoxic, affecting the neuroectoderm biomarkers PAX6 and NESTIN, and the morphology of neuronal rosettes. Corroborating the in vivo data (eye/brain defects) for one of the compounds but not for the other.

Suggesting that the ReproTracker® has potential to be used in reproductive toxicity assessment under CLP, as a way to strengthen the mechanistic knowledge and human relevance of certain toxic effects. Indicating that NAMs can facilitate a better hazard classification under CLP. NAMs screening for reproductive toxicity is essential to be able to produce chemicals that are safe and sustainable by design in the future. Despite this ReproTracker® and other similar NAMs does not currently live up to the regulatory requirements and there are several improvements needed before they are applicable in a regulatory setting.

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Development of a rapid approach for screening per- and polyfluoroalkyl substances (PFAS) in textiles

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Abstract

Textile recycling is increasingly used to help meet demands for a sustainable circular economy. However, the historical use of hazardous substances in textiles risks contaminating recycled products unless they are first removed from waste products destined for recycling. Per- and polyfluoroalkyl substances (PFAS) have been used extensively in performance outdoor textiles and are of particular concern due to their persistence, widespread environmental occurrence, and links to adverse health effects in humans and wildlife. The overall goal of this project is to develop a rapid and portable screening technique for PFAS in textiles. We started by combusting a series of commercial medical garments coated with C4- C6- or C8- side-chain fluorinated polymers. The combustion procedure converts carbon-fluorine bonds to fluoride, which is then trapped in an absorption solution. Concentrations of fluoride in this solution measured by ion chromatography (IC) were compared to those measured by two portable techniques: fluoride ion selective electrode (ISE) and colorimetry (HANNA Fluoride High Range Checker HI739). The three approaches were evaluated for accuracy, precision, specificity, and detection limits. While combustion together with ion chromatography remains the gold standard for accurate and precise screening of PFAS in textiles, the two portable techniques showed promise for the purposes of qualitative screening. Ongoing work aims to improve the accuracy of these approaches. If used in combination with a miniaturized combustion approach, ISE and colorimetry show considerable potential for rapid and portable PFAS screening in textiles.

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In Vitro Exposure of Polystyrene Nanoparticles and North Sea Marine Oil: Assessing Sensitive Endpoints of Toxicity.

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Abstract

Over the last decade, there has been increased interest in the impact of micro and nano plastic pollution on the aquatic ecosystem. However, little attention has been given to interactions of these particles with other environment pollutants found in the aquatic environment. This study aims to gain knowledge on the possible toxic effects associated with single and combined exposure of Polystyrene nanoparticles (PsNPs) and water accommodated fractions (WAF) of North Sea marine oil on fish in vitro models and explore more sensitive in vitro assays for the overall assessment of nanoparticle toxicity.

Rainbow trout (Oncorhynchus mykiss) cell lines were exposed to a range of concentrations and sizes of PsNPs alone or in combination with WAF. Endpoints measured included multitarget cytotoxicity, potential to induce CYP1A enzymes (EROD), the generation of intracellular reactive oxygen species (ROS) as well as toxicity and behavioural effects in Zebrafish embryos

Exposure to PsNPs alone demonstrated significant changes in cellular metabolic activity, membrane stability and generation of ROS in rainbow trout gill and liver cells that were observed to be particle size and concentration dependent, an increasing trend of ROS generation was observed in 25 and 100nm PsNPs exposure only and with WAF alone

Pilot studies investigating the combined exposure of similar oil: water fractions and 100nm PsNPs demonstrated reduced cytotoxicity by 25-40% at select concentrations in rainbow trout gill and liver cells. Further studies into interactions with other sizes of PsNPs will be conducted to gain an overall assessment of the risks associated with these mixtures.

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Characterizing the endocrine disrupting potential of chlorinated paraffins in vitro

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Abstract

Chlorinated paraffins (CPs) are widely used in various industries and have been measured in multiple human matrices, with exposure prevalent at all life stages. CPs are divided into three subgroups based on their carbon chain length: short (C10-13), medium (C14-17), or long (C18+). Short chained CPs (SCCPs) have been listed as persistent organic pollutants (POPs) since 2017. There are indications that some CPs have endocrine disrupting (ED) properties, but studies are limited and mainly relate to SCCPs. This study investigates the potential ED activities of a broad spectrum of CPs using several in vitro assays. The selected CPs vary in chain length and degree of chlorination, and both single and mixed compounds are included. The test battery includes assays for steroid hormone synthesis and receptor activity, retinoid signaling, and assays related to the thyroid hormone system. The androgen receptor (AR) reporter gene assay indicates that one of the CPs antagonizes the AR. Based on the TTRANSA displacement assay, the selected CPs do not bind to TTR. The retinoic acid receptor (RAR) reporter gene assay showed no receptor activity after exposure. Investigations of potential effects on estrogen receptor (ER) activity in a reporter gene assay and effects on steroid hormone synthesis, testosterone, and estradiol in the H295R steroidogenesis assay are being tested. Conclusive remarks regarding the overall ED potential await the results from the remaining assays.

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Low cytotoxicity of environmentally relevant microplastics in human lung cell models

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Abstract

Micro- and nanoplastics are ubiquitous in the environment and the air, yet health risks related to inhalation of these particles is still largely unknown. The aim of this study was to investigate the toxicity of different micro- and nanoplastics (MPs and NPs) with varied physiochemical properties. The particles included were fluorescent polystyrene (PS) particles of various sizes (50 nm, 500 nm or 7 µm) as well as aged MPs of PS, PET (polyethylene terephthalate) and LDPE (low-density polyethylene). Human bronchial epithelial cells (HBEC), macrophages (differentiated THP-1) and a co-culture of these were exposed to the different plastic particles and cytotoxicity, particle-cell interaction and ROS production were measured. The results showed that cell toxicity was seen after exposure to the spherical PS particles but mainly at high doses. The environmentally relevant (irregularly shaped, aged) MPs were non-cytotoxic. The cell dose measured for the fluorescent particles was typically around 5-10% of added dose for HBEC cells independent of size (50 nm, 500 nm or 7 µm). In contrast, it was much higher (50-65%) in the macrophages but only for the 7 µm-sized particles. Finally, marked vehicle toxicity was seen in bronchial cells but not macrophages. Vehicle toxicity may be falsely attributed to particle toxicity of commonly used commercial particles, emphasising the importance of investigating and reporting vehicle effect. This study thus suggests low cytotoxicity of MPs and NPs and highlights the need to consider vehicle toxicity and cell dose.

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Exposure to the thyroperoxidase inhibitors methimazole and amitrole disrupts testis development in rats

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Abstract

Thyroid hormone (TH) system disruption in developing rats can affect testicular growth and physiology. Studies using the thyroperoxidase (TPO) inhibitor propylthiouracil (PTU) to induce transient hypothyroidism have been associated with a reduction in testes size and changes to the testicular cell populations. While THs are known to influence testicular development, the underlying molecular mechanisms remain unclear. To address this, we investigated the effects of developmental hypothyroidism on postnatal rat testicles using two TPO-inhibitors; the pharmaceutical methimazole (MMI) and the pesticide amitrole. Pregnant rat dams were exposed to 8 or 16 mg/kg bw/day MMI or to 25 or 50 mg/kg bw/day amitrole to induce hypothyroidism. Testicles collected from 16-day old male pups were weighed, analyzed histologically, and sequenced by bulk-RNA-barcoding and sequencing (BRB-seq). Both MMI and amitrole induced hypothyroidism and testis weights were reduced. Furthermore, testis cords were reduced in diameter with an increased Sertoli/germ cell ratio, as well as an increased number of apoptotic cells. Both MMI and amitrole caused large transcriptional changes in the testes, and clustering of differentially expressed genes (DEGs) revealed a distinct pattern with almost 400 DEGs in common for the two compounds. However, amitrole exposure resulted in two additional patterns with approximately 1,200 DEGs, that were distinct from both the control and MMI groups, suggesting other direct effects of amitrole on the testis mediated by mechanisms besides hypothyroidism. These results will aid in characterizing TH-mediated mechanisms in the testis, while also providing knowledge on the hypothesized direct effects of amitrole.

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Multi-organ transcriptomics to facilitate new approach methodologies in testing for thyroid hormone system disruption

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Abstract

During perinatal life the thyroid hormones (THs) influence the development of most tissues and organs, including the brain. The TH system comprise a complex network of enzymes, transport proteins and signaling factors and hence, disruption to the system occur through various mechanisms. This may also lead to different effects in different organs depending on the mechanisms of disruption. Yet, current test methods for TH system disruptors focus primarily on the thyroid gland or circulating THs and may thus be inadequate in detecting effects of TH system disruption in other tissues. We want to address the biologic complexity and facilitate the development of new approach methodologies (NAMs) by multi-organ characterization of developing rats' exposure to TH system disruptors. In this study, under the Partnership for Assessment of Risk from Chemicals (PARC) initiative, we will use RNA-sequencing approaches to decipher mechanisms of action in multiple organs and tissues simultaneously and characterize links between apical adverse outcomes and molecular initiating events. The goal is to support an increased use of NAMs in chemical toxicity testing.

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Low-grade contaminated drinking water – a significant source of perfluoroalkyl acids (PFAA) exposure for Swedish adolescents

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Abstract

Highly contaminated drinking water (DW) have previously been suggested as important exposure sources of perfluoroalkyl acids (PFAA), yet contribution of low-grade contaminated DW (<10 ng/L of individual PFAS concentrations), has rarely been studied. We aimed to evaluate the association between the four EFSA priority listed PFAAs (Σ4PFAA), PFOA, PFNA, PFHxS and PFOS, in DW, and the association with serum PFAA concentrations in Swedish adolescents. This association was analyzed by pairing adolescent serum PFAA concentrations, from the nationally representative, school-based, dietary survey Riksmaten Adolescent 2016-17, with measured PFAA levels from the drinking water treatment plant that provided each subject with DW at home and school. The associations were analyzed using weighted least squares regression. Adolescent (ages 10-21) median serum PFNA and linear (lin)-PFHxS concentrations were <1 ng/g serum, while median lin-PFOA and lin-PFOS ranged between 1-2 ng/g serum (n=790). Median concentrations of PFAA in DW was <1 ng/L for all four PFAA. Positive associations between PFAA concentrations in serum and DW were found for all four PFAA. Adolescents exposed to Σ4PFAA concentrations above both the Swedish and Danish maximum limit in DW, i.e. 4 ng/L and 2 ng/L respectively, to a larger degree exceeded the EFSA determined health concern concentration of 6.9 ng Σ4PFAA/mL in serum compared to participants with DW concentrations below the maximum limits. Our results suggest that low-grade PFAA contamination of DW significantly contributes to adolescent bodyburdens. More research is needed to understand consequent health implications at such low exposure levels.

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Training the next-generation toxicologists

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Abstract

The master's programme in toxicology (ToxMaster) at Karolinska Institutet was one of the first toxicology programmes world-wide. During these 47 years, the competency requirements for toxicologists has changed and thus, the content of this programme has continuously been updated in line with the developments in science and regulations. Today, as the next-generation toxicology and risk assessment focus on New Approach Methodologies (NAMs) that are coming into use, while traditional methods are still required, the current students need knowledge and skills in all types of methodologies. The KI ToxMaster continuously follows developments through close connections to frontline research, regulatory toxicity testing and risk assessment activities, which is important for keeping a high standard in all areas included in the programme. This includes collaborations with experts (often ToxMaster alumni) at universities, regulatory agencies and industry. Especially important for the inclusion of NAMs, Adverse Outcome Pathways (AOPs), Integrated Approaches to Testing and Assessment (IATAs), QSAR and the 3Rs has been the regular study visits to the EC Joint Research Centre and EURL-ECVAM since 2004. Since 2010 the ToxMaster is a global programme admitting 25-30 students each year for this 2-year programme. More than 600 students from about 30 countries worldwide have graduated since 1976, and 60 students are currently within the programme. The ToxMaster is providing well-trained toxicologists competent in both established and new methods. Thus, ToxMaster alumni contribute to the transition of toxicology and risk assessment in their roles as researchers and risk/safety assessors at agencies, industry and consultancy companies.

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Toxicity of real-world road tunnel emissions in an ALI exposure model.

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Abstract

Fine particle matter generated by fuel combustion can become harmful when inhaled because these particles are small enough to reach and deposit at the deepest part of the lungs. Different studies investigate the toxicity of filter-collected particles, but few study the airborne particles in a real-world environment.

In this study, a mobile air-liquid interface (ALI) system was used to test the toxicity in real time to fresh fine particles emitted from vehicles driving in an urban road tunnel in the center of Stockholm. The ALI system contains human lung cells (A549) and was used to mimic particle inhalation into the lungs. During experiments, the ALI system was placed behind the wall next to the road tunnel. Cells were exposed for two hours to road tunnel emissions concentrated 5 times. Cells were then investigated for cytotoxicity and inflammatory response after 24h incubation.

Cell viability was not affected in any of the groups (road tunnel air without particles, exposure to particle emissions) compared to the negative control. A biological increase of inflammatory markers IL-1β, IL-8, and IL-6 was observed in cells exposed to road tunnel emissions compared to filtered tunnel air and negative controls. No statistical significance was observed for any of the measured cytokine due to the variation of exposure dose between experiments in a real-world environment.

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Chemical activity as an approach to evaluate phytoplankton sensitivity to PAH mixture

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Abstract

This project aims to investigate the sensitivity of different phytoplankton species to chemical activity. Saturated solutions of acenaphthene, fluorene, phenanthrene and fluoranthene were prepared in methanol and combined to produce mixtures in a range of chemical activities (0.01 – 0.15). Chemical activity and passive dosing were integrated into an algae toxicity test, where silicone was loaded with the PAH mixture. The effect of growth inhibition on five phytoplankton species (Rhodomonas salina , Phaeodactylum tricornutum , Monoraphidium minutum , Prymnesium parvum and Picocaryote species) was assessed. Both medium and biota samples were analyzed through LC and GC-MS methods for exposure confirmation. Biota samples were collected for total lipids and lipid profiling. The results from Rhodomonas salina show that the normalized concentrations of PAHs in the algae converted to chemical activities using organic carbon-water partition coefficients (Koc) were within the equilibrium range. Chemical activity and growth inhibition followed a dose-response curve, and the effective activity that caused 50% effect was 0.078, which agrees well with earlier studies that demonstrated biological effects to occur at chemical activities of 0.01-0.1. Chemical activity caused a decrease in photosynthetic efficiency in R. salina . The remaining phytoplankton species were exposed to the same PAH mixture where P. parvum was the most sensitive and P. tricornutum the most tolerant species. This study provides confirmatory evidence that the chemical activity concept can be used to assess the mixture effects of Hydrophobic Organic Chemicals (HOCs) on the toxicity test. This is an ongoing experiment, and the lipid characterization is being evaluated for further comparisons.

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Developing an Adverse Outcome Pathway from androgen receptor antagonism to hypospadias

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Abstract

Adverse Outcome Pathways (AOPs) can help chemical risk assessors by defining causal links between initial molecular stressor events and adverse outcomes in intact organisms. Within the AOP structure, key events along the causal pathway are connected through evidence-based key event relationships (KER). The KERs are the units of knowledge for which causality can be inferred, and thus AOPs can be used for mechanistic-based predictive toxicology. To fully exploit the potential of the AOP framework, AOP networks must be built and KERs properly developed. Currently, there are no AOPs described for hypospadias, a genital malformation affecting up to 1/125 newborn boys. Developmental exposure to endocrine disruptors has been associated with hypospadias in humans, experimentally shown in animals, and is assessed for in reproductive toxicity guideline studies, making such AOPs highly relevant. We are developing an AOP linking androgen receptor (AR) antagonism to hypospadias (AOP 477). To establish a non-adjacent KER between reduced AR activity and hypospadias, we conducted a semisystematic literature search in PubMed and WoS. A total of 1,319 titles and abstracts were screened using pre-defined exclusion criteria, resulting in 84 articles for full text review and 211 human case studies as additional evidence. The reliability of animal studies will be evaluated using the online tool SciRAP, and the weight of evidence for the KER will be assessed. This KER will be an essential part of the evidence for AOP 477 and included in future AOPs for hypospadias, starting from stressor events such as disrupted steroidogenesis and inhibition of 5α-reductase.

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Transcriptomics of rat thyroid glands following developmental exposure to thyroperoxidase inhibiting chemicals reveal distinct profiles

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Abstract

Thyroperoxidase (TPO) is a key enzyme in thyroid hormone (TH) biosynthesis. Many chemicals can inhibit TPO, resulting in decreased TH levels and in vivo effects similar to hypothyroidism. However, the causal pathway between TPO inhibition and in vivo adverse outcomes are not well described at the molecular level. Filling this data gap could allow for identification of sensitive biomarkers. Here, we have performed detailed transcriptomics profiling of the thyroid gland from male rats developmentally exposed to three in vitro TPO inhibitors: amitrole, MBI and cyanamide. Pregnant rat dams were exposed from gestation day 7 to pup day (PD) 16. Serum TH concentrations were measured at PD16 from male rat offspring to verify decreased TH levels and thyroid glands were collected for transcriptomic analysis by bulk-RNA-barcoding and sequencing. Amitrole induced a distinct transcriptional profile, which largely reflected the severity of effects observed in vivo . This unique transcriptional profile was associated with tissue growth and remodeling. The greatest intersection of differentially expressed genes were identified between amitrole and MBI, supporting previous findings of disrupted TH system in vivo for these two substances. Interestingly, only few TH-synthesis genes were affected. Possibly, the identified group of intersecting genes between amitrole and MBI can serve as biomarkers of in vivo TPO-inhibition; however, further studies should investigate the applicability and sensitivity of this transcriptional signature.