The Belgian Society for Toxicology and Ecotoxicology

Annual Meeting 2022

Current State of Knowledge on the Toxicology and Ecotoxicology of PFAS

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Abstract Book

The Belgian Society for Toxicology and Ecotoxicology

Annual Meeting 2022

Abstracts Invited Speakers

PFAS Chemicals and their Applications

Pierre Vander Elst, 3M Belgium bv, Zwijndrecht, Belgium

It is the intention of this presentation to make people more familiar with the chemistry aspects of per- and polyfluoroalkyl substances (PFAS). First, an overview will be given of the definitions and scope of PFAS by ECHA and OECD, and the PFAS restriction proposal of ECHA (REACH).
Subsequently, the physico-chemical properties of fluorochemicals that are important for the commercial products will be presented followed by the description of production processes and applications of these fluorochemicals.
The PFAS addressed will be monomers of fluoropolymers, fluoropolymers (fluoroelastomers, fluorothermoplastics, PTFE), polymerization production aids, fluorotelomers (telomer building blocks and polymers), perfluoropolyethers, the Simons electrochemical fluorination process for the production of perfluorocarbons, “sulphonamide” based fluorochemistry, hydrofluoroethers, fluoroketones and fluoronitrile. The applications linked to this broad variety of PFAS chemicals will mainly focus on their uses in industrial processing, automotive industry, electronic & electrical industrial applications.

Ecological Exposure and Effects of PFAS

Thimo Groffen, Department Biology, UAntwerpen, Belgium

Per- and polyfluoroalkyl substances (PFAS) are a large, heterogeneous group of anthropogenic chemicals of potential concern to human health and the environment. Based on the information of a few relatively well-studied PFAS, such as PFOS and PFOA, it is known that a subset of PFAS can be considered persistent, bioaccumulative, and/or toxic. Multiple strategies and regulations have been implemented to reduce the production, emissions and use of specific PFAS. However, manufacturers have often replaced these legacy PFAS with new PFAS or chemical substitutes.
The environmental fate and behaviour of PFAS differ from neutral lipophilic organic compounds, giving implications regarding traditional bioaccumulation metrics. Still little is known on the environmental fate of many PFAS, including the alternatives to PFOS and PFOA. Despite the significant increase in the volume of research publications identifying PFAS in environmental media and wildlife, knowledge on exposure sources for wildlife (and humans) is still limited. In addition, suitable toxicity data for risk assessment is currently still lacking.
In this presentation, I will summarize the current understanding of PFAS exposure in wildlife, effects of phase-out and regulations on PFAS concentrations in the environment and biota, and currently available data relevant to PFAS toxicity in wildlife. I will present both examples from literature as well as our own research findings. Finally, I will describe the critical gaps and uncertainties regarding wildlife exposure and toxicity.

PFAS and Health: a Toxicological Perspective

Greet Schoeters, Department Biomedical Sciences & Toxicological Centre, UAntwerpen, Belgium

Perfluoralkylated substances (PFAS) are ubiquitous in the environment. They constitute a large chemical class that contains a few thousand substances. Humans are exposed by contaminated water, by PFAS that enter the food chain, by inhaled air and air dust and by contact with PFAS that are present in a wide diversity of consumer goods. All over the world, long chain PFAS are detected in serum of almost all humans that have been tested. Over the years, studies with experimental animals have demonstrated a variety of adverse health effects of mainly PFOS and PFOA. Human studies comprise workers in PFAS producing plants, residents of contaminated areas and longitudinal studies. PFOA and PFOS serum levels are increasingly associated with health outcomes. New insights, mainly from the human data, but backed with experimental data and some mechanistic insights have urged risk assessment committees to lower the limit values. The presentation will provide an overview of the current state of knowledge that pushed to lower limit values, the new studies that go beyond, the remaining gaps of knowledge and the challenges to deal with risk assessment of this large substance group.

EU policy on perfluoroalkyl substances (PFAS) in food and feed

Frans Verstraete, European Commission, DG for Health and Food Safety, Brussels.

The EU legislation on contaminants in food fulfils two essential objectives: the protection of animal and public health and removal of internal barriers to trade within the EU.
Following the principles and objectives of the General Food Law, feed and food safety legislation shall pursue a high level of animal and human health protection. To achieve this objective legislation shall be based upon risk analysis. Risk assessment shall be based on the available scientific evidence and undertaken in an independent, objective and transparent manner. Risk management shall take into account the results of risk assessment, other factors legitimate to the matter under consideration and the precautionary principle where appropriate.
When international standards exist or their completion is imminent, they shall be taken into consideration in the development of any standard at EU level
In the framework Regulations on contaminants in feed and food it is foreseen that
- contaminant levels shall be kept as low as can reasonably be achieved by following good practices at all stages of the production chain
- feed and food placed on the EU market shall be safe in order to protect animal and public health, maximum levels for specific contaminants shall be established where necessary;
- the consultation of a scientific body (EFSA) for all provisions which may have an effect upon animal and public health is mandatory.
Maximum levels are established at a reasonably achievable level, stimulating feed and food business operators to apply preventive actions all along the feed and food chain in order to avoid the contamination of the feed and food chain.
Taking into account the outcome of the EFSA opinion Risk to human health related to the presence of perfluoroalkyl substances in food, published in September 2020. EFSA established a Tolerable Weekly Intake (TWI) of 4.4 ng/kg bw per week for the sum of Perfluorooctane sulfonic acid (PFOS), Perfluorooctanoic acid (PFOA), Perfluorononanoic acid (PFNA) and Perfluorohexane sulfonic acid (PFHxS) on the basis of the effects on the adverse effects on the immune system. EFSA concluded that the exposure of parts of the European population exceeds the TWI, which is of concern. Maximum levels are established in certain foods for PFOS, PFOA, PFNA, PFHxS individually and for the sum of these 4 PFAS. In addition, a Commission Recommendation (EU) 2021/1431 has been adopted recommending to monitor PFAS from 2022-2025 in a wide range of foods. Commission Implementing Regulation (EU) 2022/1428 establishes methods of sampling and analysis for the control of maximum levels for PFAS in certain food. For feed, it is important to develop sufficient sensitive methods of analysis and to ensure sufficient capacity before elaborating on a monitoring recommendation and in a later stage possible maximum levels.

PFAS (PFOA/PFOS) Exposed Workers: Is there Sufficient Evidence to recommend Extended Health Surveillance?

Perrine Hoet, UCLouvain, FEDRIS, presented by Mark Martens, FEDRIS, Brussels, Belgium

PFAS producing industry has published in peer-reviewed scientific journals the results of studies relying on the data of voluntary medical surveillance programmes. These data mainly concern the levels of serum biochemistry parameters. For instance, increased total cholesterol and low density lipoproteins, triglycerides, liver enzymes or thyroid hormones, have been associated with jobs with PFOA/PFOS exposure in some studies but not in others. In studies where positive associations are shown, the level of the health effect was found to be small.

Regarding the few studies on disease incidence or mortality, the analyses are limited by the qualitative nature of the exposure assessment, the lack of consideration of important confounders, and the too small size of the cohort to detect differences in disease incidence or mortality. There is a lack of consistency in findings between studies. Examples are an increased risk in cerebrovascular disease and prostate cancer. An increased risk was suggested in a mortality study among PFOS exposed workers, but the extension of this study to identify incident bladder cancers in this occupational cohort found little support for such association.

Impaired vaccine response, which was regarded by some international and national agencies, as the most consistently reported association in adults in the high-exposure community population and in studies of children in the general population has not been studied in occupationally exposed workers. There is also no data regarding toxicity to reproduction and foetal development in the occupational context.

Biomonitoring studies also objectified exposure to PFAS in other occupational sectors, such as firefighters and ski waxers, but at much lower levels. The potential association between such exposure and health effects has been little studied.

In conclusion, there is no current evidence that supports a significant impact of PFOS/PFOA on workers' health. In particular, the evidence of an increased risk of specific diseases that would justify a prolonged health surveillance programme is inadequate at this time. Therefore, the FEDRIS commission on chemical agents recommends a longitudinal epidemiological study on workers, who, on the basis of a risk assessment, could be exposed to PFOS/PFOA via inhalation and/or dermal exposure to levels exceeding those of the general population (e.g. manipulation of contaminated soil).

Comparative Toxicology of two Perfluoroalkyl Sulfonates

Sue Chang, Corporate Occupational Medicine, 3M Company, St. Paul, Minnesota, United States

As science of the various perfluoroalkyl and polyfluoroalkyl substances (PFAS) continues to develop, perfluorooctane sulfonate (PFOS), a long-studied long-chain perfluoroalkyl sulfonate, anchors various points of research investigations and regulatory activities. Due to the structural similarities, equal toxicity characterization (relative to PFOS) has sometimes been assumed when it comes to other perfluoroalkyl sulfonates, such as perfluorobutane sulfonate (PFBS), a short-chain perfluoroalkyl sulfonate. Even within the same class, different perfluoroalkyl sulfonates have different physical/ chemical properties arising from the chemistry of the carbon-fluorine bond that differentiate their biological activity, including toxicokinetics, tissue dosimetry, mode of action, and toxicology profiles. This presentation will compare and summarize the general findings from the laboratory toxicology studies between PFBS and PFOS.

The Role of PPAR-alpha in the Toxicity of HFPO-DA

Shawn Gannon, The Chemours Company, New Castle, Delaware, United States

Hexafluoroproylene oxide dimer acid (HFPO-DA) is a short-chain polyfluoroether used by Chemours as a polymerization aid. Although widely referred to as a "PFOA replacement", this is not accurate. The use scenario for HFPO-DA by Chemours is limited to polymerization aid for some types of fluoropolymers. It is not a commercial product for Chemours, and so it is not sold into potentially environmentally dispersive markets like firefighting foam.

Like for many PFAS compounds, the liver is the most sensitive target organ identified in repeated-dose toxicity studies of HFPO-DA in rats and mice. The mechanism of action (MOA) behind this observation is of importance because not all adverse effects observed in rodents are relevant to humans. Using non-human relevant endpoints to derive human health values like Reference Doses or Human Equivalent Doses leads flawed and conservative assessments.

The weight of evidence from transcriptomics studies performed with HFPO-DA indicates that this substance exerts its effect exclusively via the nuclear receptor Peroxisome Proliferator-Activated Receptor alpha (PPAR-alpha). Although PPAR-alpha is present in human cells, there are fundamental differences between how rats and mice and how humans respond to PPAR-alpha activation and the liver histopathological effects observed in these two rodent species are not relevant to humans. There is a wealth of knowledge about the effects of PPAR-alpha activators in humans because this receptor is the pharmaceutical target for the fibrate family of drugs. The fibrates have been used safely for over 50 years to reduce triglycerides and raise high density lipoprotein (HDL) cholesterol, the so-called "good" cholesterol.

Because many studies with PFAS compounds have identified the liver as the key target organ, and effects like increased liver weights are consistently observed, it is likely that other PFAS are also mediated by a PPAR-alpha MOA. Available data shows that the MOA of HFPO-DA is exclusively PPAR-alpha but some other PFAS may also activate additional nuclear receptors, like CAR or PXR. The transcriptomics approach used with HFPO-DA can aid in the identification of MOA and allow for grouping based on those MOA.

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Abstracts Young Scientist Competition
Oral Presentations

Platelet Activation by Antisense Oligonucleotides (ASOs) in the Göttingen Minipig, including an Evaluation of Glycoprotein VI (GPVI) Ontogeny

Allan Valenzuela, Miriam Ayuso, Laura Buyssens, Chloé Bars, Chris Van Ginneken, Steven Van Cruchten

Comparative Perinatal Development, Department of Veterinary Sciences, Faculty of Pharmaceutical Sciences, Biomedical and Veterinary Sciences, University of Antwerp, Wilrijk, Belgium

Antisense oligonucleotides (ASO) are a therapeutic modality that enables selective modulation of undruggable protein targets. However, dose- and sequence-specific reductions of platelet counts have been reported in nonclinical studies and clinical trials arising from various molecular mechanisms. The adult Göttingen Minipig is an acknowledged nonclinical model for the safety testing of ASOs, and the juvenile Göttingen Minipig has been recently proposed for the safety testing of pediatric medicines. However, no data on the platelet effects of ASO sequences and modifications are available for Göttingen Minipigs. This study assesses the effects of various ASO sequences with different modification patterns on Göttingen Minipig platelets using in vitro platelet activation and aggregometry assays. The underlying mechanisms are also investigated to further characterize it as a model for ASO safety testing. In addition, the abundance of glycoprotein VI (GPVI), which was previously found to interact with ASOs in adult human samples, was investigated in adult and juvenile Göttingen Minipigs. Our data on direct platelet activation and aggregation by ASOs (2'-O-methoxyethyl and locked-nucleic acid phosphorothioated (PS) oligonucleotides) in adult Göttingen Minipigs are remarkably comparable to human data and serve as a key risk indicator for thrombocytopenia. Additionally, PS ASOs bind to platelet collagen receptor GPVI and directly activate Göttingen Minipig platelets in vitro, mirroring the findings in human blood samples. This further corroborates the use of the Göttingen Minipig for the safety testing of ASOs. Moreover, the abundance of GPVI protein in juvenile Göttingen Minipigs provides an insight into the influence of ontogeny in potential ASO-induced thrombocytopenia in pediatric patients.

Innate-like B-1 Cells collaborate with Macrophages for Pulmonary Particle-induced Granuloma Formation

Léa Hiéronimus · Raïssa Demazy, Laura Christiaens, Francine Uwambayinema, Jean Francois Geuens, Yousof Yakoub, François Huaux

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Inhalation of persistent inorganic particles trigger certain cells to form compact cellular aggregates to contain the foreign body. The formed structures, granulomas, can mature into fibrotic granulomas associated with excessive extracellular matrix remodeling. The current paradigm for explaining lung granulomatous diseases induced by inhaled particles is mainly based on activated macrophages. This mechanism is now challenged because additional immune cells systematically infiltrate and affect exposed tissue. We previously demonstrated that the absence of B cells leads to decreased lung granulomatous fibrosis in silica-treated mice. Here, we investigated the role of B lymphocytes and demonstrate that among B cells, innate-like B-1 cells (not conventional B-2 cells or plasma cells) were specifically accumulated in the lungs of mice instilled with micrometric particles, during the development of granulomas. Instillation with particles that are not granuloma-inducing further illustrated that B-1 cells respond specifically to granuloma-inducing particles. We than used in vitro models to determine how B-1 cells respond to inorganic particles in the lung environment. In comparison to macrophages, purified B-1 cells show resistance to the cytotoxicity of particles and clustered them for establishing cell/particle aggregates in vitro. B-1 cells (not B-2 cells) in co-culture with M2 (not M1) macrophages and particles organized three-dimensional cell granulomas in Matrigel and the release of TIMP-1. These effects were specific to particles that are granuloma inducing. In conclusion, our data indicate that, beside macrophages, B-1 lymphocytes participate in micrometric particle-induced granuloma formation in conjunction with macrophages. During this project, we established novel in vitro models for fundamental and predictive toxicology.

The Bioavailability, Bioaccumulation and Biomagnification of PFAS in a Simplified Terrestrial Food Chain

Jodie Buytaert

Department Biology, UAntwerpen, Belgium

The emergence of PFAS around the world has resulted in an environmental contamination of these compounds in many different biota and even humans.
Recently there has been a rising concern about the rate of bioaccumulation and -magnification of PFAS within terrestrial food webs, because there are still many uncertainties about the behaviour of these compounds within such food webs.
Once present within the lower trophic levels of the food chain, some PFAS can biomagnify, resulting in higher concentrations in organisms at higher trophic levels. Inter-species differences in exposure sources might result in differences in PFAS accumulation among species. Because of the many uncertainties, research needs to be conducted on the accumulation potential of PFAS in the different terrestrial biota, as well as on the differences among different matrices.
The present study investigated the bioavailability and bioaccumulation of PFAS in a simplified terrestrial food chain near and on the domain of 3M, a known PFAS hotspot. The great tit was chosen to occupy the highest trophic level. Other matrices e.g. soil, nettles and different invertebrate taxa were also investigated. Secondly, the effects of PFAS on different enzymatic and non-enzymatic oxidative stress biomarkers were assessed in the red blood cells of the great tits.
The present study was able to find evidence for the bioaccumulation of PFAS within this simplified terrestrial food chain. The different matrices accumulated different PFAS in varying degrees. Species-specific differences were observed among the herbivorous, detritivorous and carnivorous invertebrate species. These differences likely reflect their differences in PFAS exposure through different sources. The study was not able to find significant associations between elevated PFAS concentrations and the different (non-)enzymatic biomarkers in great tits, in contrast with a previous study performed. This suggests that elevated PFAS concentrations do not necessarily lead to oxidative stress, but it could still contribute to this. A possible explanation for the not seen effects could be that the measured PFAS concentrations did not exceed the PFOS no observed adverse effect level (NOAEL) reference values. Due to the remaining uncertainties, there is a need for a continuous monitoring of the exposure to these PFAS as well as the effects on populations.

Zebrafish early Life Stages in the Study of Metabolic Disruption: The Case of Paraoxonase

Rik van den Boom[1], Lucia Vergauwen[1], Katyeny Manuela da Silva[2], Alexander van Nuijs[2], Adrian Covaci[2], Dries Knapen[1]

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Endocrine disrupting chemicals (EDCs) have been extensively investigated for their disruptive effects on steroid hormone pathways. Recent studies have shown that some EDCs can also disrupt energy metabolism, which could result in adverse health effects, and is commonly referred to as metabolic disruption. Currently, the underlying mechanisms are not fully understood and there is an urgent need for assays to identify metabolic disruptors. In this study, we used zebrafish early life stages, both larval and embryonic, to study metabolic disruption, and we focused on the potential role of paraoxonase 1 (PON1) in the underlying mechanisms. PON1 is an enzyme with antioxidant functions, known to be associated with metabolic disorders such as fatty liver in humans and to metabolize xenobiotic substances. In the first assay, effects of metabolic disruption on PON1 were investigated in 2-week old zebrafish larvae in different metabolic conditions. Metabolic disruption was induced by a 5-day feeding regime with a custom western diet and exposure to different doses (200 µg/L or 2000 µg/L) of a metabolic disruptor, bisphenol A (BPA). Changes in dietary energy contents resulted in a non-significant increase of weight, length and oxidative stress. The addition of BPA exposure on top of the dietary metabolic shifts resulted in a significantly increased weight, length, and oxidative stress, indicating that the metabolic disrupting effects of BPA further exacerbated the metabolic changes induced by the Western diet. Pon1 activities also increased in the same manner as the metabolic changes, showing a link with increasing metabolic disruption. Our data showed a significant correlation between whole body pon1 activities and oxidative stress. In conclusion, pon1 likely performs part of the antioxidant response to the increasing oxidative stress caused by metabolic disruption. In a second assay, we investigated which effects indicative of metabolic disruption can already be measured in a zebrafish embryo until the age of 5 days, an assay that is considered a new approach methodology (NAM) as alternative to animal testing. Zebrafish embryos were exposed from 2 to 120 hours post fertilization to lipid metabolism disrupting compounds, including BPA, the Peroxisome proliferator-activated receptor γ (PPARγ) agonist rosiglitazone and the PPARγ antagonist T0070907. So far, effects were observed on morphology and yolk size. The embryonic assay can quickly identify metabolic disruptors using a NAM approach, while the larval assay allows for a more in-depth, metabolically relevant study of metabolic disruptors in interaction with a dietary component.

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Abstracts Young Scientist Competition Posters

Y1
Are Accumulated PFAS Concentrations in Aquatic Macroinvertebrates related to the Ecological Quality of Water Courses?

Cara Byns, Thimo Groffen, Lieven Bervoets

Department Biology, UAntwerpen, Belgium

Despite the detection of alarmingly high PFAS concentrations in terrestrial environments across Belgium, data on the impact of PFAS in aquatic ecosystems is scarce.
Aquatic macroinvertebrates play a key-role in freshwater ecosystems by driving ecosystem services such as nutrient cycling, sediment mixing and energy flow through food webs. Because some taxa are more sensitive to pollution than others, macroinvertebrates can act as bioindicators for pollution. Whereas sediment and water samples represent only a momentary pollution status and do not consider differences in bioavailability driven by fluctuating abiotic and biotic factors, the macroinvertebrate community structure can expose the cumulative impact of short- and long-term pollution events. We pose that water and sediment quality criteria might not always be adequate for the protection of aquatic communities and should be complemented with data on biotic indicators.
The aim of this research is to study the relationship between accumulated PFAS concentrations in three benthic invertebrate species (Chironomus sp., Asellus sp. and Gammarus sp.) and the ecological status of aquatic ecosystems. Therefore, resident invertebrate taxa are collected at 32 sites in rivers and water courses across Flanders, Belgium. To assess the invertebrate community responses, the ecological water quality index, i.e. the Multimetric Macroinvertebrate Index of Flanders (MMIF), is calculated. Simultaneously, sediment and water samples are collected to investigate possible relationships between accumulated PFAS in the biotic and abiotic environment. Based on the accumulated concentrations in resident taxa we aim to derive threshold body burdens of PFAS for the protection of the aquatic ecological status.

Y2
A realistic PFAS Mixture severely disrupts Zebrafish Development

Gustavo Guerrero-Limón, Marc Muller

ULiège, Liège, Belgium

The European Union is moving towards a more conscious way of living. Initiatives such as the European Green Deal comprise a set of actions and proposals to tackle several major issues at a regional level, ranging from climate crisis, energetic transition, food safety, etc. Within such initiative, special chapters have been devoted to specific chemicals. This is the case of the per and poly fluoroalkylated substances with particular attention to the perfluorooctanesulfonic acid (PFOS). The latter is a man-made Persistent Organic Pollutant (POP) whose properties have deleterious effects that may lead to decreased fertility, increased high blood pressure in pregnant women, increased risk of some types of cancer, interference with the immune system, amongst many others.
In our study, we exposed zebrafish larvae for minimum 96 hours to a POPs mixture and a sub mixture of PFAS, both containing PFOS. These POP and PFAS mixtures were assembled according to what was found in blood samples of Scandinavian people. Thus, its formulation and concentrations are environmentally- and medically relevant. Though a lethal dose was established at a really-unlikely-to-happen high dose, a more realistic sub-lethal dose was still inducing remarkable deleterious effects. We found that the standard length was significantly reduced, for instance. The exposure affected cartilage and bone development. We also estimated the cardiotoxicity potential and found that fish had an increased heart rate. For those individuals where the exposure did not induce obvious morphological aberrations, their swimming behaviour was tested. This endpoint was also affected, in a nutshell, exposed fish were hyperactive in comparison to the controls. Though PFOS is a well-known chemical for the responses it induced in other organisms, our results suggest that the power of PFOS might be enhanced when administered within a mixture. The variety of responses recorded add up to the already existing catalogue of defects caused by such relevant chemicals.

Y3
DMSO Concentrations up to 1% are Safe to be used in the Zebrafish Embryo Developmental Toxicity Assay

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[2]Natural Products and Food Research and Analysis (NatuRA), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, University of Antwerp, Wilrijk, Belgium.

Dimethyl sulfoxide (DMSO) is a popular solvent for developmental toxicity testing of xenobiotics in zebrafish embryos and larvae, as it appears to be less toxic in this model than other well-known solvents. High concentrations of DMSO, however, are toxic for zebrafish embryos and larvae. Therefore, it is recommended to keep the final DMSO concentration as low as possible, preferably not exceeding 100 µL/L (0.01%). Yet, higher concentrations of DMSO are often required to dissolve non-water soluble compounds in an aqueous medium. Therefore, the aim of this study was to determine the highest concentration of DMSO that can be safely used in our standardized Zebrafish Embryo Developmental Toxicity Assay (ZEDTA). In the first part of this study, zebrafish embryos were exposed to different concentrations (0%, 0.01%, 0.1%, 0.5%, 1% and 2%) of DMSO. No significant increase in lethality or gross morphology malformations were observed when using DMSO concentrations up to 1%. In a follow-up experiment, we assessed whether two compounds that cause no developmental toxicity in the ZEDTA, ascorbic acid and hydrochlorothiazide, remain negative when dissolved in 1% DMSO. The combination of 1% DMSO and the compounds caused no significant increase in lethality or gross morphology malformations. In conclusion, DMSO concentrations up to 1% are safe to use to dissolve compounds in the ZEDTA.

Y4
Bioavailability of PFAS in Homegrown Eggs is Associated with Soil Concentrations and Soil physicochemical Characteristics

Robin Lasters, Thimo Groffen, Marcel Eens, Lieven Bervoets

Department Biology, UAntwerpen, Belgium

Poly- and perfluoroalkyl substances (PFAS) can enter the human food chain due to their persistence, widespread use and global distribution. Particularly, consumption of self-cultivated food, such as homegrown eggs, has been related with increased human exposure to PFAS. However, very little is known about the local factors driving the exposure in laying hens and hence the potential accumulation in eggs. Soils often form the basis within the human food chain and are known to be a PFAS sink. While sorption processes of PFAS have been frequently studied, very little is known on its implications to the bioavailability of these pollutants in the human food chain. Furthermore, the relationship of PFAS with soil characteristics has rarely been studied in real-life field settings.
Therefore, the main objectives of this study are to examine the role of multiple soil characteristics and concentrations on the bioavailability of PFAS in homegrown eggs and whether these variables can predict PFAS concentrations in this food matrix. To this end, soil and egg samples were collected from 91 private gardens across Flanders. These matrices were analyzed for 32 target PFAS and the following soil characteristics were measured: total organic carbon (TOC) content, cation-exchange capacity (CEC), pH, electrical conductivity and clay content.
Results showed that most of the variation in egg PFOS and PFOA concentrations was explained by the corresponding soil concentrations and TOC content. Both higher soil concentrations and higher TOC contents were associated with higher egg concentrations for PFOS and PFOA, although the effect size of these predictors was higher for PFOS compared to PFOA. The CEC was negatively associated with both PFOS and PFOA egg concentrations, while clay content was positively related with PFOS egg concentrations. Together, the present results suggest that the soil can be an important direct and indirect PFAS exposure source to free-ranging laying hens. Cross-validation of the final regression models indicated that soil concentrations and characteristics could adequately predict egg PFOS concentrations and to lesser extent PFOA egg concentrations. However, training the model with additional field data is necessary to validate the model's predictability.
Further research should include potentially other exposure sources to hens, such as soil invertebrates (earthworms) which live in close relation to the soil matrix, to disentangle the associations between the egg concentrations and some of the soil characteristics. Also, they may explain additional variation of the egg concentrations and hereby further improve the predictability of the models.

Y5
Exposure to Silica: what does Biomonitoring tell us?

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Respirable crystalline silica (RCS) is widely present in several industries and settings. Millions of workers worldwide are exposed to this mineral and have a higher risk of developing fibrosis, bronchitis and cancer. Robust epidemiological studies have also reported a significant correlation between occupational exposure to silica and systemic autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and systemic sclerosis (SSc). While it has always been thought that silica particles remain strictly confined within the lungs after inhalation, the development of silica-induced autoimmunity suggests that a possible fraction of biopersistent silica particles may migrate into the peripheral lymph nodes and activate systemic immune responses. To evaluate the possible particle dispersion, we developed a new method for tissue mineralization (NaOH treatment) and silica quantification (ICP-MS). A biomonitoring of silica exposure was established by measuring silicon and metallic tracer levels in organs of C57BL/6 mice instilled intratracheally with DQ12 silica and sacrificed at short- and long-time points. We also intend to measure silicon levels and markers of autoimmunity in serum samples of patients included in a Belgian cohort of systemic sclerosis.

Y6

Poorly Soluble Low Toxicity (PSLT) Particles modulate Genes related to Inflammation and Cancer Development to a greater Extent in Rat than in Mouse Alveolar Macrophages

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Poorly soluble low toxicity (PSLT) particles, e.g. titanium dioxide (P25) and carbon black (CB) can induce chronic inflammation and lung cancer in rats at inhalation exposure levels leading to lung particle overload. Overload is associated with altered alveolar macrophage function and mobility, and although PSLT overload has been observed in several experimental species (rats, mice, hamsters, monkeys), the rat appears as the only one that develops chronic inflammatory and carcinogenic lung responses. Whether or not these rat responses can be expected in humans exposed to PSLT particles remains a source of debate and has led to the classification of PSLT as possible human carcinogens. In order to clarify human hazard it is important to understand first how PSLT overload can lead to adverse lung outcomes in rats. This study was thus designed to identify possible mediators and pathways of PSLT carcinogenicity in rats by comparing the responses of rat and mouse alveolar macrophages to PSLT overload.

Method

Primary alveolar macrophages from rats and mice were exposed in vitro to P25 or CB particles at doses leading to non-overload or overload conditions. Four days after exposure, untargeted transcriptomic analyses were performed to assess and compare gene expression under the different conditions and species.

Results

In mouse macrophages, there were no significantly differentially expressed genes at overload conditions, compared to control and non-overload conditions, after either P25 or CB particles. In rat macrophages, respectively 197 and 300 genes were significantly differentially expressed at overload conditions of P25 and CB particles, and 32 were common to both PSLT particles. Among these 32 genes, some were relevant for overload (impaired alveolar macrophage mobility and activity), inflammation or cancer development. Rat/mouse comparison of the variation in expression of the differentially expressed genes in rats revealed that genes related to overload tended to vary in the same direction for both rat and mouse macrophages, whereas genes related to inflammation and
cancer development tended to vary differently and to a greater extent in rat macrophages.

Conclusion

These in vitro observations are consistent with the in vivo studies, highlighting the fact that rats and mice respond differently to PSLT overload and that genes related to inflammation and cancer development are modulated to a greater extent in rat alveolar macrophages than in mouse macrophages.

Y7
Comparison of BMD-derived Genotoxic Potencies of Mycotoxins Co-occurring in Food and Feed

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Mycotoxin contamination in European regions is expected to increase in the near future due to global warming and globalization of food markets. Since multiple mycotoxigenic fungi can colonize the same food or feed material and are able to produce different mycotoxins simultaneously, the co-occurrence of mycotoxins and the associated mixture toxicity, even at low concentrations, has raised concerns for human and animal health. Moreover, several of these mycotoxins are suspected to be genotoxic compounds and little is known about their possible interactions in case of co-exposure. For non-genotoxic endpoints, the principle of additivity is assumed to generally apply, although other types of combined effects have been described as well. In order to investigate whether the principle of additivity is justifiable for genotoxic mycotoxins, relative potencies of individual compounds need to be evaluated followed by mixture testing. To this end, six suspected genotoxic mycotoxins known to co-occur in maize were selected including both regulated and emerging mycotoxins. The genotoxicity of these compounds was tested in the in vitro micronucleus assay in TK6 cells, both in the presence and absence of S9 metabolic fraction. The benchmark dose approach was next applied in order to determine the relative genotoxic potencies. Preliminary results obtained with aflatoxin B1, deoxynivalenol and zearalenone demonstrate that in the absence of S9 metabolic fraction deoxynivalenol and zearalenone are equally potent, while AFB1 is less potent. Data obtained from this potency ranking were further used to demonstrate how self-made mixtures can be composed in assessing the combined effects of co-occurring mycotoxins.

Y8

The Neonatal Göttingen Minipig as Translational Model for Perinatal Asphyxia and Therapeutic Hypothermia

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Introduction. Animal models can be useful tools to better understand the mechanisms of conditions and therapies in the paediatric population, such as perinatal asphyxia (PA) and therapeutic hypothermia (TH). In this respect, we used the neonatal Göttingen Minipig as translational model. We hypothesized that PA, as well TH, have an impact on drug disposition in the neonatal Göttingen Minipig. Midazolam, fentanyl, phenobarbital and topiramate were selected as model compounds due to their clinical relevance. In vitro and in vivo animal experimental data were collected to support future dose precision of these medications in patients. For this purpose a non-survival experimental protocol was developed and used. Methods. 4 conditions i.e., hypoxia (group H), therapeutic hypothermia (group TH), hypoxia + hypothermia (group H+TH) and controls (group C), with 6 piglets per condition, were investigated. Within 24h of partus, ~600 g healthy male Göttingen Minipigs were sedated with Zoletil® 100 (Tiletamina/Zolazepam), followed by induction of anaesthesia with Isoflurane. Mechanical ventilation was performed and central and peripheric venous catheters were inserted for continuous infusion and drug administration of the 4 model compounds, as well for blood sampling. Hypoxia was induced by setting the inspiratory oxygen fraction (FiO2) at 15%, using nitrogen gas. Results. Study procedures were well tolerated for 24h in 24 neonatal Göttingen Minipigs of 551.12g (± 60.32g). Central venous catheterisation proved to be the best method for vascular access. Peripheric catheterisation was easiest in the epigastric vein, whereas catheterization of the umbilical vein depended on whether the umbilical cord was still wet or not. Cooling was easy to control and to maintain at a target rectal temperature of 33.5°C. Hypoxia could be established for 51 (±34.82) min. Increased blood lactate 9.56 (±2.27) mmol/L and decreased pH 7.00 (±0.16) were used as key parameters for PA. The impact of hypoxia and hypothermia on the plasma concentrations of midazolam, fentanyl, phenobarbital and topiramate is still under investigation. *Conclusions*
This study, in which the neonatal Göttingen Minipig model was subjected to a setting comparable as in a human NICU, was successful. We showed that PA can be induced in neonatal Göttingen Minipigs, and the effect of PA and TH on drug disposition can be studied separately, for 24h. These data reveal the potential of the neonatal Göttingen Minipig as promising nonclinical in vivo animal model in safety assessment for conditions in the human paediatric population.

Y9
Developmental Effects caused by Micro- and Nanoplastics in Schmidtea Mediterranea

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Plastics are indispensable in our daily lives. They are used in various applications like packaging, personal care products and medical equipment. Due to a lack of proper waste management and disposal, plastic degrades to micro- and nanoplastics (MNPs), less than 5 mm and 100 nm respectively, and ends up in the aquatic environment. So far, it is known that these particles can cause hazardous effects in aquatic organisms. However, in-depth knowledge of the developmental effects and their relationship with the physicochemical properties is still lacking in current risk assessment strategies. In this study, the effects on development were assessed using the freshwater
planarian, Schmidtea mediterranea, as a model organism. Because of the large amount of pluripotent adult stem cells, S. mediterranea is capable of regenerating all body tissues, including the nervous system. This makes it possible to study developmental effects in an in vivo model organism in line with the 3 R principles and REACH regulations. S. mediterranea was exposed to carboxylated (COOH) polystyrene particles of 50 nm, 215 nm, 1 µm and 2 µm. The physicochemical properties of the particles were characterized using transmission electron microscopy (TEM) and dynamic light scattering (DLS). Particle uptake was studied using confocal microscopy. All particles were taken up regardless of their size and had a size and concentration dependent effect on general regeneration, eye development and the formation of the anterior commissure in the brain ganglia. Underlying these results cell cycle dynamics were affected and a decreased trend in stem cell division and proliferation was observed. We hypothesize that mitochondrial redox dynamics modulate the particle induced responses. Our results highlight the importance of linking physicochemical characteristics to induced toxicity mechanisms, which is the way forward for more complete risk assessment strategies.

Y10

The Mechanistic Linkage between Thyroid Hormone System Disruption and Developmental Neurotoxicity due to Bisphenol Exposure in the Zebrafish Embryo

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The developing central nervous system is more vulnerable to environmental insults than the adult one due to critical developmental processes (e.g. synaptogenesis, migration, etc.). Exposure to neurotoxicants during childhood can therefore contribute to a variety of neurodevelopmental and neurological disorders, of which the prevalence increases over the years. However, information on the toxic effect of these chemicals on neurodevelopment is limited. Research in rodents shows how developmental neurotoxicity (DNT) endpoints (e.g. impairment of memory and learning or decrease in cognitive function) can be linked to thyroid hormone system disruption (THSD). For example, a decrease in hippocampal function could potentially lead to previously mentioned adverse neurological endpoints. In fish however, evidence of THSD-induced DNT is fragmented and the mechanisms and impacts are unclear. Since neurotoxicants can be found in the aquatic environment, it is key to gather knowledge on the adverse effects these compounds have. In this research, the adverse effect on the neuro-endocrine system in the zebrafish embryo will be determined in order to study the mechanistic linkage between THSD and DNT. Zebrafish embryos are commonly used in toxicology testing and are considered a new approach methodology (NAM). The focus of this research is on bisphenols: bisphenol A (BPA), bisphenol F (BPF) and bisphenol S (BPS) will be tested and they can be found in the aquatic environment (e.g. waste water, sediment and surface water) due to their use in industry. BPA is a man-made chemical that is commonly used to manufacture polycarbonate plastics and resins and can therefore be found in food containers and linings for food and beverage cans, among other things. Due to evidence on its toxicity (e.g. interfere with hormonal system and adversely affect reproduction) and its ability to leach into foods and beverages, the use of BPA was limited by the European Commission (0.05 mg/kg). Hence the production of bisphenol analogues in industry became popular. BPF and BPS are commonly used analogues but research has proven that they can also have endocrine disrupting properties. It is therefore important to determine their adverse effect on the neuro-endocrine system. This study will provide an overview on the available knowledge of the adverse effects of these compounds, as well as relevant endpoints for DNT testing in the zebrafish embryo. Preliminary research on mortality and locomotion after exposure to BPA, BPF and BPS will also be shown, together with their expected toxicity based on quantitative structure-activity relationship (QSAR) modelling.

Y11
Epigenetic Alterations Induced by Occupational Exposure to Hexavalent Chromium

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The occupational exposure of workers to hexavalent chromium (Cr(VI)) occurs in various industries, such as the chrome plating industry, welding industry and the industry comprising surface treatment with chromates. Inhalation exposure to Cr(VI) is associated with increased risk of lung cancer. The mode of action for Cr(VI)-induced lung cancers is well described, however the exact mechanisms underlying the carcinogenesis of Cr(VI) are not well elucidated. At the mechanistic level, both genomic instability and epigenetic modifications induced by exposure to Cr(VI) have been proposed.
The HBM4EU chromates study investigated, among other things, the effect of Cr(VI) exposure on oxidative stress, global DNA methylation and global DNA hydroxymethylation in blood. The study population consists of Cr-platers (n = 57), surface treatment workers (n = 46), welders (n = 110) and controls (n = 99). Post-shift urine samples and blood samples were collected at the end of the working week. Metal concentrations were measured with ICP-MS. Analysis of methylation, hydroxymethylation and oxidative stress levels were done using LC-MS/MS.
The highest Cr(VI) exposure levels were observed for Cr-platers. The mean (± SD) urinary total chromium levels in post-shift urine samples per exposure activity were 2.5 (± 2.8) µg/g creatinine for chrome plating workers, 1.3 (± 1.2) µg/g creatinine for welders, 1.7 (± 1.8) µg/g creatinine for surface treatment workers and 0.35 (± 0.33) µg/g creatinine for controls. Furthermore, these platers were exposed to elevated levels of PFAS. Significant differences between workers with chromium exposure and controls were found for the perfluoroalkyl sulfonic acids, particularly for perfluorooctylsulfonic acid (PFOS). The partly high PFOS exposure in chromium platers can be explained by the former application of PFOS as fume suppressants in electroplating baths.
Regarding epigenetic alterations, global DNA hypomethylation was observed. Furthermore, increased levels of oxidative stress was observed. These results suggest that DNA methylation may be influenced by oxidative stress.

Y12
The Role of Mitochondria in Nanoparticle-specific Stem Cell Toxicity

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Humans increasingly encounter nanoparticles (NPs) in everyday applications such as face masks, personal care products and foodstuff but their potential health effects are still largely unknown. Current risk assessment strategies are insufficient since they lack in-depth information on the relation between uptake, toxicity and the physicochemical properties of NPs. In addition, there is a large knowledge gap regarding the developmental effects. The present study examines the mechanisms underlying particle-induced toxicity of different NPs in human dental pulp stem cells (DPSCs). DPSCs are used as a proxy for mesenchymal stem cells and developing tissues, in line with the 3R principles and REACH regulation. Cell cycle dynamics, and related redox and mitochondrial parameters, were investigated in relation to the NP characteristics and kinetic profiles. We compared three widely implemented NPs, namely polyvinylpyrrolidone-coated silver (PVP-Ag), titanium dioxide (TiO2) and carboxylated polystyrene (COOH-PS) NPs. The studied NPs were characterised using dynamic light scattering and transmission electron microscopy (TEM). Cellular uptake and localisation in endosomes and in some cases the cytosol was confirmed using TEM and confocal microscopy. PVP-Ag NPs and TiO2 NPs were cytotoxic and decreased cell proliferation at 10-50 µg/ml and 100-250 µg/ml respectively, depending on the duration of exposure. COOH-PS NPs did not significantly affect cell viability at the concentrations tested (up to 250 µg/ml). Next, cell counts and cell cycle phase distributions confirmed that only PVP-Ag and TiO2 NPs affect cell cycle dynamics, although differently. While PVP-Ag NPs induced cell cycle dynamics for some concentration and duration combinations, exposure to TiO2 NPs resulted in inhibition after longer exposure to 100 µg/ml TiO2. Underlying these changes exposure induced mitochondrial swelling, membrane depolarization and
network structure alterations as determined by TEM, JC-10 assay and Mitotracker CMXRos Red imaging, respectively. The effects varied with the exposure dose. Furthermore, absence of endoplasmic reticulum dilations and presence of lipid droplets were detected via TEM after 48 h exposure to PVP-Ag and TiO2 NPs. Our preliminary results suggest that TiO2 NPs and mostly PVP-Ag
NPs harm stem cell functioning while COOH-PS NPs do not show marked adverse effects. We hypothesise that mitochondria operate as a signalling hub, guiding the activation of downstream transcriptional processes and stem cell dynamics.

Y13
Development of a Method of Embedding and High-throughput Histology of 8-day old Zebrafish (Danio rerio) Larvae

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The need for high-throughput histological analysis methods of large numbers of zebrafish (Danio rerio) larvae came up to meet the increasing demand for zebrafish as a reference model in the laboratory. This method has become a useful tool in the study of modern clinical medicine, histopathology and research. Because of the small size of zebrafish larvae, it is valuable to develop an accurate method to perform histological sections of many larvae simultaneously in the same cutting plane. This reduces the laboratory work but also the time needed to identify individual changes. Here we describe the details of a method for embedding and sectioning histological slides using larval matrices. This work aims to optimize a histological method to detect changes and deleterious effects in tissues, especially in the digestive tract, of 8-day old zebrafish under simultaneous exposure to microplastics and a bacterial pathogen (Aeromonas hydrophila). High-quality histological images will give us useful information on how early exposure to microplastics can affect the response and resistance of zebrafish larvae to an infectious agent. Until today, there is no standardized or commercialized solution for this type of analysis, it must be developed and manufactured in the laboratory. The mold developed in our laboratory is designed to perfectly fit the shape of an 8-day old larvae to facilitate the inclusion process and allows to set 24 larvae per slides. In this way, we managed to obtain good quality and reproducible sagittal histological slides where the digestive tract and other tissues are clearly visible.

Y14
Towards an improved understanding of how thyroid hormone system disruption affects the swim bladder in zebrafish

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Thyroid hormones (THs) are critical for normal vertebrate development and thyroid hormone system disruption (THSD) has therefore been linked to many different adverse effects. Recently, an adverse outcome pathway (AOP) network has been described linking THSD to impaired swim bladder inflation. In zebrafish, the swim bladder consists of two chambers: an anterior and posterior chamber, which inflate during different phases in development. During embryonic development, inhibition of TH activation (conversion of thyroxine to triiodothyronine by deiodinase enzymes), leads to impaired posterior swim bladder inflation. The anterior chamber, which inflates during the larval stage, is affected by inhibition of TH activation as well as inhibition of TH synthesis. In the current study, we used deiodinase inhibition as a tool to advance our mechanistic understanding of how THSD affects the posterior chamber. Zebrafish embryos were exposed to iopanoic acid, a model deiodinase inhibitor, during different phases in swim bladder development. Three different phases in swim bladder development can be identified: budding, pre-inflation phase and the inflation phase. The budding and pre-inflation phase mark the formation of the swim bladder and its three structural tissue layers. During the inflation phase, the embryos gulp air to inflate the swim bladder. Impaired swim bladder inflation was observed following exposure during the budding and pre-inflation phase. Exposure during the inflation phase did not affect the swim bladder. This shows that the formation and not the inflation of the posterior swim bladder is directly affected by deiodinase inhibition. This was further confirmed by gene transcription analysis. Significant decreases in mRNA levels of key genes in swim bladder formation, such as hedgehog signaling and markers of the different tissue layers, were found. TH measurements were carried out and showed that there was no endogenous TH synthesis yet during the time period when deiodinase inhibition affects the swim bladder. The results of this study demonstrate that deiodinase inhibition directly impacts processes underlying swim bladder formation, and that these processes rely on maternal rather than endogenously synthetized THs. Knowledge gained in this research can be extended to other adverse effects of THSD besides the swim bladder. As it is difficult to identify endpoints specific for THSD, characterizing the life-stage specific sensitivities of different endpoints, might allow for assembly of a THSD response profile. This could in turn be used in the further development of THSD screening methods.

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F1
Distribution and Bioaccumulation of PFAS in the Aquatic Environment: A Mesocosm Study on PFAS Uptake from Sediment by an Aquatic Plant

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Per- and Poly Fluorinated Substances (PFAS) are a widely used group of anthropogenic compounds, with a multitude of industrial and consumer applications. The widespread use of PFAS, together with their persistence and bioaccumulation potential, has led to global contamination of the abiotic and biotic environment. Nevertheless, their distribution across environmental compartments, including potential uptake by plants, remains largely understudied. Therefore, we investigated the distribution and bioaccumulation of PFAS from sediment to an aquatic plant in a mesocosm study. To this end, sediment cores originating from a heavily PFAS-contaminated (Blokkersdijk pond, Antwerp) and an urban reference site (lake Gaasperplas, Amsterdam) were sampled. Shoots from the cultured, rooting macrophyte Myriophyllum spicatum were planted and left to grow in these sediments with overlaying Dutch Standard Water (DSW) under laboratory conditions. After 56 days, the distribution and bioaccumulation of PFAS with different molecular structures was determined in all matrices. We screened for 7 different PFAS, including 3 isomer pairs (L-PFOS, Br-PFOS, L-PFOA, Br-PFOA, L-PFHxS, Br-PFHxS, PFBS, FBSA, 6:2 FTS, PFECHS). All selected PFAS were detected in the sediment from the contaminated site, while 6 out of 10 were also present in the reference sediment. All but one compounds were detected in the roots and shoots of the plants that grew on the contaminated sediment, while 5 were detected in the roots and shoots of the plants that grew on the reference sediment. The calculated Biota-to-Sediment Accumulation Factors (BSAFs) for the 3 isomer pairs, showed that linear isomers had a higher bioaccumulation potential from the sediment to the roots of the plants compared to the branched isomers. It is concluded that PFAS are widely present in aquatic environments and that their bioaccumulation potential is driven by their molecular structure.

F2
Exploring the Taxonomic Domain of Applicability (tDOA) of Thyroid Hormone System Disruption using the Adverse Outcome Pathway Framework

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Multiple studies have associated the exposure to chemicals like bisphenols, polychlorinated biphenyls (PCBs) or Per- and polyfluoroalkyl substances (PFASs) with endocrine disruption, and specifically thyroid hormone system disruption (THSD), in humans and wildlife. Current regulatory efforts focus on developing fast and efficient screening methods to identify thyroid hormone system disrupting compounds in the environment. These efforts comprise the substitution of animal-based assays, mainly rodent studies, by new approach methodologies (NAMs) such as zebrafish or amphibian embryo assays and thus increase future needs for cross-species extrapolation. Adverse outcome pathways (AOPs) can support this need by providing causal linkages from molecular initiating events (MIEs; describing the interaction between a chemical and a molecular target) to adverse outcomes (AOs; on individual or population level) via key events (KEs; describing measurable, altered biological states).
Different AOPs for THSD have been developed which are, in most cases, applicable to a single taxon. They do, however, share multiple KEs and thus form a THSD AOP network that connects e.g., AOPs developed in fish with AOPs described in mammals. Making use of this network, we evaluated whether paths leading, for example, to impaired learning and memory in mammals, are potentially applicable to other vertebrate taxa. In doing so, we aimed to identify paths with a high potential for cross-species extrapolation that could further support the development of tailored, alternative (non-mammalian) test methods.
Assessing the taxonomic domain of applicability (tDOA) of MIEs and AOs in the THSD network was achieved by means of a literature study. In this study a special focus was on the link between MIEs and AOs with altered thyroid hormone levels as these present key elements in the THSD network, linking almost all AOPs with each other. Our meta-analysis suggests that a majority of MIEs is applicable across taxa. Further, we found that paths in the network leading, for example, to adverse neurodevelopmental outcomes, possess a high potential for cross-species applicability, while paths leading to thyroid cancer are most likely applicable to rats only. Based on this analysis we are now able to prioritise paths suitable for cross-species extrapolation and highlight the potential of using data from non-mammalian vertebrate classes to predict THSD-induced effects in mammals, including humans, and vice versa.

F3
PTFE presents a low Risk to Human and Environmental Health and differs substantially in its Hazard Profile from other Types of PFAS

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The term per- and polyfluoroalkyl substances (PFAS) encompasses a wide and diverse array of chemistry containing at least one fully fluorinated carbon. Recognizing that PFAS are not all the same, it is critical to assess the hazards/ risks of PFAS based on their potential to impact human health or the environment and do not justify being managed as one class. Fluoropolymers are a unique type of PFAS that enable highly beneficial products while having low human health and environmental hazards.
This work presents physical, chemical, toxicity, stability and leachable analytical data on a high molecular weight, fine powder polytetrafluoroethylene. This data demonstrates: stability under environmental conditions suitable to life, the lack of leachables, the lack of transformation to perfluoroalkyl acids or other non-polymeric PFAS substances of potential concern, low toxicity, a history of safe clinical use in medical devices, and effective approaches for destruction. The capability to produce and dispose of this PTFE responsibly will be discussed to represent the full lifecycle of this very valuable polymer. This work highlights the low risk of the fluoropolymer type of PFAS, especially when compared to other types of PFAS, and the need to avoid unintended restriction of use of these highly beneficial substances.
Regulators should address PFAS in a proportionate risk-based manner and prioritize resources on those PFAS that exhibit properties such as: water solubility (mobility), bioavailability, the potential for a substance to bioaccumulate, toxicity, and the propensity for a substance to degrade into other substances of concern. These properties are most often associated with the non-polymeric category of PFAS.

F4
Stimulating the Regulatory Acceptance of a novel Platform developed for the Assessment of Chemical-induced Cardiotoxicity

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Humans are continuously exposed to a wide variety of potentially toxic chemicals including per- and polyfluoroalkyl substances (PFAS) [1]. Several adverse health outcomes, including the increasing occurrence of cardiovascular diseases (CVD), have been linked with environmental exposure to exogenous toxic chemicals [2]. However, cardiotoxic effects of environmental pollutants and their co-exposures with drugs in the vulnerable population (such as older people) are not well understood. Furthermore, cardiotoxicity is still not addressed as a separate endpoint in current risk assessment strategies although many chemicals have been shown to manifest cardiotoxic properties [3],[4]. In this context, the project ALTERNATIVE has been initiated.

Within the Horizon 2020 project ALTERNATIVE (grant no. 101037090), a novel platform will be developed to detect the cardiotoxicity of chemical mixtures [5]. The platform will consist of a 3D in vitro model mimicking the healthy and aged human cardiac tissue, coupled with high-throughput multi-omics analyses, and integrated into a Machine Learning (ML) risk assessment tool. One of the key objectives of the ALTERNATIVE project is to stimulate the regulatory acceptance of this novel platform to provide a more robust basis for decision-making.

Integrated Approaches to Testing and Assessment (IATAs) have been proposed as science-based methodologies for chemical hazard characterization. They usually start with an integrated analysis of all existing toxicological information, such as data from computational models, traditional in vivo, ex vivo and in vitro testing. The outcome of this analysis defines if and which new approach methodologies (NAMs) shall be applied as a second step to complement available data for supporting regulatory decision making [6]. Today, NAMs are not yet widely and consistently used for regulatory decision-making due to the limited insights in the mechanisms understanding toxicity. The Adverse Outcome Pathway (AOP) framework can be applied to characterize mechanistic relevance of NAMs in predicting an adverse outcome. Thus, AOPs can be used as a framework to develop IATAs [7].

In this work, we first identified the current regulatory data requirements for cardiotoxicity outlined in the European Commission (EC) regulations and guidance for industrial chemicals, pharmaceuticals, pesticides, and biocides. Furthermore, the available epidemiological and toxicological evidence for cardiotoxic chemicals and environmental pollutants was reviewed. Currently, mechanistic insights in cardiotoxicity induced by environmental pollutants and chemicals are collected by using the AOP framework. In a next step, an IATA for cardiotoxicity will be drafted based on the developed AOPs according to the international accepted paradigm [8]. The outcome is expected to provide the outline of a potential practical regulatory approach to assess the cardiotoxic potential of chemicals and environmental pollutants, taking into account existing data sources, models, and NAMs for cardiotoxicity.

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F5
Monitoring Microplastics in Ikopa River (Madagascar)

Andry Ny Aina Rabezanary

Microplastics are ubiquitous in the aquatic environment. Once released, they can float in the water column, or end-up by colonizing the aquatic sediment. This study aims to monitor the microplastics therein the Ikopa river, which deserves the city of Tananarive (Capital of Madagascar) as a drinking water and an agricultural purpose. During sampling, superposed sieves with sizes between 5 mm and 50 µm were used to collect the microplastics. From upstream to downstream of the city, 3 zones were respectively chosen (Zone 1: near to the source, zone 2: at the exit of the industrial zone, zone 3: at the exit of the city), and 3 sampling periods were set depending on the season (S1: the end the dry season – S2: the rainy season) – S3 : the start of the dry season). As a result, microplastics fragments with range size from 100–500 µm were dominant, up to 42.506±16.353% (mean±sd) and followed by fibers (24.695±12.242%, mean±sd). Additionally, microplastics concentration was significantly higher during the rainy season compared to the dry season (207.333±28.014 particles/m3 , mean±sd) where most of the aquatic population carry out their breeding activity.

Further analysis will be made concerning the microplastics characterization, such as a Raman microspectroscopy (µ-Raman) to identify polymer's chemical compositions, a scanning electron microscopy (SEM) to assess the surface morphology and microorganism's colonization of the microplastics, metagenomic analysis to assess the microbial profile, and GC mass spectrometry to determine adsorbed pollutants attached from the environment.

Microplastics accumulation in fish tissues (gills and digestive tract) will be also assessed as we had performed fish sampling at the upstream and downstream of the river.

This study brings a new insight concerning the microplastics monitoring in African freshwater.

F6
Design and Application of a Semi-automated Workflow including In Silico Models to support the Risk Assessment of Food Contact Material Substances

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In order to support the safety assessment of food contact material (FCM) substances, a semi-automated workflow was developed to collect toxicological information in a fast and efficient way. The workflow combines existing toxicological data with (quantitative) structure-activity relationship ((Q)SAR) model predictions and is particularly relevant for non-regulated FCM substances for which no or few toxicological data are available like non-intentionally added substances (NIAS) and substances migrating from non-plastic FCM. First, existing databases and/or lists of substances containing toxicological information were identified. In addition to our in-house database with FCM-related information, these include the European Food Safety Authority (EFSA) OpenFoodTox database, the ECHA database as well as the SIN list and lists of compounds with (suspected) endocrine disrupting (ED) activity. Next, (Q)SAR models to predict toxicological endpoints relevant for FCM were selected from the open-access VEGA. The endpoints of interest were identified based on the data requirements described in ESFA's Note for Guidance for FCM substance safety assessment and include genotoxicity, sub-chronic toxicity (NOAEL), bioaccumulation potential, developmental and reproductive toxicity and carcinogenicity. Moreover, models for ED activity and to identify the Cramer class as part of the threshold of toxicological concern (TTC) strategy were also selected. Within VEGA, each prediction provided by a particular model is accompanied by a value indicating the level of reliability of the prediction, i.e. the applicability domain index. Currently, a software is being developed that allows to easily collect the relevant information from the databases of interest and to combine it with the prediction outcomes of the (Q)SAR models, using the Simplified Molecular Input Line Entry Specification (SMILES) as the main input entry. Afterwards, the applicability of the workflow will be investigated by means of different case studies on non-evaluated compounds present in FCM and known to migrate into food.

[1]EFSA CEF Panel (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids), 2008. Note for Guidance for the preparation of an application for the safety assessment of a substance to be used in plastic Food Contact Materials. EFSA Journal 2008; 6(7):21r, 41 pp. https://doi.org/10.2903/j.efsa.2008.21r.

F7

The RE-Place Open Access Database provides Insight into the use of New Approach Methodologies in Belgium

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Although the 3Rs Principle (Replacement, Reduction, Refinement) was introduced more than six decades ago, the replacement of animal testing remains a challenge in the 21st century. Innovative technologies can help to reduce or sometimes even avoid the use of experimental animals in the life sciences. They are referred to as 'New Approach Methodologies (NAMs)' and include, amongst others, sophisticated cell- and tissue cultures, computer modeling, high throughput testing and '-omics' technology. Even though the importance of NAMs is gaining interest worldwide, their international acceptance and (regulatory) implementation is often lagging behind. Stimulating the development and use of NAMs may benefit from a bottom-up approach, i.e. local initiatives mapping the available expertise on NAMs and promoting their use. An example of such an initiative is the 'RE-Place' project. Due to the fast development of NAMs, (young) scientists may encounter difficulties in finding relevant, reliable and up-to-date information. The RE-Place open access database facilitates the search for information by providing an extensive overview of the existing expertise on NAMs in Belgium. All submitted NAMs are linked with the names of experts and organisations where the technologies have been developed and/or are currently applied. This type of information is often not readily available to all stakeholders involved (scientists, ethical committees, regulators, the government, etc.). By having access to a direct point of contact, networking activities are strongly encouraged and facilitated, allowing to speed-up the development and validation processes which are needed for the implementation of NAMs.

Experts can easily submit their know-how on NAMs via the RE-Place online tool. The knowledge is then automatically integrated in the RE-Place open access database, available via www.RE-Place.be. In October 2022, the database contained 201 methods submitted by 138 experts from 24 different (research) institutes. The majority of the submitted methods is situated in basic and applied research and is categorized as 'in vitro or ex vivo'.

Overall, the RE-Place project promotes the use of NAMs by sharing (inter-)nationally available knowledge via the website and social media channels. Face-to-Face meetings are organized with experts from various research areas to present the project and set-up new collaborations. This approach ensures a close and reliable interaction with all involved stakeholders. As such, the RE-Place project not only helps to raise awareness, but also builds bridges and increases trust in the use of these new technologies, thereby stimulating the regulatory uptake of NAMs and their daily use.

F8
Fun with NAMs!

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Education brings neither glory to the scientists nor a better h-index. Nevertheless, it is useful for multiple reasons such as knowledge sharing, capacity building and creation of an adequate ecosystem. Overall, one can admit that the education and training about 3Rs at university level has the merit to exist even if it could be possibly better advertised and communicated. The JRC launched a mapping exercise on this matter in 2018 but as far as the authors are concerned the results of the study were not published (1). A category of individuals that is rarely targeted properly is the general public as well as teaching at primary and secondary school. JRC took care of the latter by providing learning scenarios to empower the teachers (2). Moreover, organising open days as well as participating in science festivals are great venues for reaching out the general public. Still, there is space for creativity by providing other formats. At Altertox, two new concepts and formats are expected to complement the current "arsenal" of tools available. The first one is an edutainment game meant to open a conversation about NAMs (New Approach Methodologies) and validation process in a fun and convivial environment. “TATABOX” (Towards Alternatives To Animal testing) tiles are not meant to be exhaustive in terms of content as well as persona but rather a starting point for discussion with concrete items within a team on the process towards regulatory acceptance. The second one is a quiz game for youngsters (from 9+, “Little genius”) meant to raise public awareness about NAMs, laboratory animals, life sciences, legislation in Europe and NAMs job. Questions for quiz were adapted to general public using true/false, multiple choices, open ended questions. Hopefully, these two new formats will provide supplementary ways for the scientific community to exchange at national level.

(1) https://joint-research-centre.ec.europa.eu/jrc-news/education-and-training-3rs-2018-02-27_en
(2) Introducing the Three Rs into secondary schools, universities https://publications.jrc.ec.europa.eu › JRC123343