Annual Meeting 2024 – Poster Session

Poster 01

Characterization of a rainbow trout brain cell line for developing a cell-based model in environmental neurotoxicity testing

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Chemicals released into water bodies can affect the neurophysiology of aquatic organisms, endangering their survival. Traditional environmental risk assessment relies on time- and resource-consuming animal experiments.

Here, we propose rainbow trout (*Oncorhynchus mykiss*) brain cells (RTbrain) as an animal-free alternative for the toxicity assessment of neuroactive pollutants. The presented research aims to develop a set of RTbrain cell-based assays covering different aspects of neurotoxicity.

Firstly, RTbrain cells are characterized by establishing baseline transcriptome and proteome profiles, studying structural properties by immunocytochemistry and functional capabilities by electrophysiology. While multi-omics data are still being processed, studies on gene expression and immunocytochemistry have revealed the presence of neuronal-specific features. The molecules identified include markers for glial cells, neurons, and neuronal progenitor cells, indicating heterogeneity within the cell line culture. Preliminary electrophysiology studies have identified potential electrical activity which has to be further verified.

These results strengthen the potential of RTbrain to be used for the development of an *in vitro* test battery test, which will be established according to the neurotoxicity targets and endpoints identified in the cell line.

Keywords: environmental neurotoxicology, alternatives to animal testing, fish cell lines, *in vitro* test battery

Poster 02 (selected for Short Oral Presentation)

Effects of two propylene glycol ethers on pulmonary gas diffusion: a human and *in vitro* study

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Propylene glycol monomethyl ether (PGME, CAS # 107-98-2) and propylene glycol butyl ether (PGBE, CAS # 5131-66-8) are organic solvents used in a plethora of occupational and household products, such as cleaning products, paints, degreasing, and inks. As volatile organic compounds, PGME and PGBE can be inhaled by users during domestic and professional tasks. Occupational and non-occupational pulmonary dysfunctions and diseases have been reported upon organic solvent exposures but a causal link with specific chemicals is lacking. In a preliminary study we observed a darkened blood color in healthy participants following inhalation exposure to PGME and PGBE, suggesting a possible decrease in oxygen supply from the lungs. The effects of these chemicals on pulmonary gas diffusion were then evaluated in healthy participants (n=11) exposed 4-hours to PGME (35 ppm) or PGBE (15 ppm) under controlled inhalation exposure conditions. Capillary blood gas measurements (O_2 saturation, partial pressure of O_2, and hemoglobin states) were performed before, during, and after exposure. Toxic effects of PGME and PGBE on alveolar membrane functions (surface tension, surfactant production, and cell permeability) were assessed with a 3D human alveolar tissue model under air-liquid interface conditions. Our results showed a significant decrease in blood oxygenation levels in our exposed participants. In vitro exposures revealed alterations in alveolar membrane functions, with an increase in surface tension and a decrease in cell permeability, which could explain the reduced diffusion of gas observed in the participants. By integrating both human & *in vitro* approaches, we revealed a link between PGME and PGBE exposure and pulmonary effects. Our findings highlight the need for protective measures to safeguard the health of individuals routinely exposed to these solvents by inhalation, particularly in occupational settings.

Keywords: oxygen diffusion, glycol ethers, human inhalation exposure, lung cell effects, toxicology

Poster 03

Toxicity and cellular trafficking of functionalized nanoparticles for drug delivery through the Blood-Brain Barrier

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Nanoparticle-mediated delivery of therapeutics is a promising method for treating central nervous system (CNS) disorders. The effectiveness of this approach strictly relies on the ability of the nanocarriers to cross the blood-brain barrier (BBB) while protecting the potency of the carried therapeutics and minimizing potential toxicities to the BBB. It is essential to thoroughly under-stand the mechanisms by which nanocarriers are internalized, transported within cells and re-leased into the CNS.

Here, we produced silica nanoparticles (SNPs) targeting the BBB using combinatorial chemistry. Transferrin and D-glucose were used as Trojan horses to promote particle internalization via transferrin receptor and/or the glucose transporter 1, highly expressed on the luminal side of the brain endothelial cells (BMECs). The influence of SNPs surface functionalization on cell viability, barrier integrity and different endocytic pathways was investigated using the human cell line, hCMEC/D3. Mechanisms influencing transcytosis were addressed using endocytosis inhibitors (chloroquine, methyl-beta-cyclodextrin and BAY 876) and uptake competitors (free transferrin).

Produced SNPs were not toxic to the cells and did not affect their barrier tightness. The attachment of transferrin and D-glucose promoted SNPs uptake through clathrin-mediated endocytosis. On the contrary, non-functionalized SNPs were internalized via caveolae and clathrin-independent endocytosis as confirmed by their decreased uptake in cells pretreated with methyl-beta-cyclodextrin. The attachment of transferrin and D-glucose resulted in SNPs accumulation in cells pretreated with chloroquine. Moreover, it reduced the lysosomal trapping of SNPs, an essential requirement for successful exocytosis.

In summary, combinatorial chemistry is an effective approach to generate and evaluate several SNPs surface modifications. Nanocarrier optimization enhances cellular uptake, advancing the delivery of therapeutic agents to the target CNS area. Moreover, *in vitro* assessment can help evaluate any potential adverse effects on the BBB.

Poster 04

Cytotoxicity of *Helleborus foetidus* extracts in cancer cell lines and primary cells.

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Anthroposophic medicine can be used to broaden therapeutic options, supplementing conventional pharmacological interventions. The aim of this study was to investigate the anti-cancerous potential of *Helleborus foetidus* extracts that could be used as an anthroposophic drug for integrative oncology treatment. Plant extracts were prepared from vegetative and generative parts of plants harvested either in summer or in winter. A selection of cancer cell lines and healthy cells were used to assess the effects of the extracts in terms of viability, migration, changes in cell cycle, and induction of apoptosis. To gain insights about the differences in chemical content of different plant extracts and to identify active compounds of *H. foetidus*, samples were analyzed by LC-MS and specific fractions tested for their toxicity.

The results show that the extracts were cytotoxic to the cancer cell lines, albeit with different potency and efficacy. Interestingly, healthy, primary human fibroblasts and keratinocyte were highly sensitive to the extracts, whereas mouse fibroblasts were more resistant. Analysis of the fractions obtained from the extracts demonstrated that *H. foetidus* contains numerous bufadienolides, which were main drivers of the observed cytotoxicity (specifically for the human cells).

In conclusion, the use of a panel of cancer and healthy cells, coupled with analytical methods allowed us to identify the most potent substances present in *H. foetidus* extracts. Based on the results, these extracts have the potential to be developed as an anthroposophic phytopharmaceutical drug to support integrative cancer therapies. However, attention will need to be paid to potentially harmful effects on healthy cells.

Poster 05

Adapting to Global PFXS Regulations: Impact Assessment in the Medical Device Industry

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Per- and polyfluoroalkyl substances (PFXS) are a group of synthetic organic compounds used in numerous applications, including textiles, packaging, lubricants, refrigerants, electronics, and medical devices. PFXS and their metabolites are highly persistent substances, and if not controlled, their concentrations in the environment will continue to rise. Due to this persistence, PFXS remediation is both difficult and costly. Additionally, certain PFXS compounds are known toxicants and bioaccumulate, posing significant risks to human health.

Currently, several legislative updates and regulatory initiatives are underway to address PFXS contamination. In the USA, the Environmental Protection Agency (EPA) has introduced new drinking water regulations for six PFXS compounds. In the EU, the European Chemicals Agency (ECHA) has published a universal restriction proposal targeting around 10,000 substances, including PVDF and PTFE fluoropolymers. ECHA's scientific committees for risk assessment (RAC) and socioeconomic analysis are currently evaluating the impacts of this proposal. While certain uses may receive derogation periods, most stakeholders will have just 18 months to comply with the new regulations. If approved, this would mark the first time such a broad restriction is applied. Without immediate action from manufacturers, these regulations will significantly impact various industries and limit market access to potentially critical products.

This analysis focuses on the potential impact of these upcoming restrictions on the medical device industry. Manufacturers are advised to assess alternatives and thoroughly understand their supply chains to identify and mitigate potential risks. In addition, the changes may require the re-evaluation, restructuring, or revalidation of manufacturing processes, facilities, and products. Moreover, increased analytical and reporting requirements, along with enhanced waste management responsibilities, will add further legal obligations for medical device manufacturers.

In summary, the forthcoming PFXS regulations will reshape the medical device industry. To continue providing safe and effective products, manufacturers must proactively assess, strategize, and comply with these new requirements.

Poster 06

Strategy to characterize the toxicity of the emerging mycotoxins Beauvericin and Moniliformin

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Beauvericin (BEA) and Moniliformin (MON) are emerging mycotoxins produced by fungi of the genus Fusarium. They constitute a public health concern due to their natural occurrence in crops and feeds and possible adverse effects on humans and animals. For BEA, these include potential neurotoxic and myotoxic properties as well as endocrine-disrupting (ED) activity. Regarding MON, cardiotoxicity was identified as its main adverse effect in pigs, turkeys and chickens. Although these mycotoxins are frequently detected, no legislation or guideline is available, and toxicity data is limited.

To simulate the systemic distribution of BEA and MON in mice, rats and humans, we used Physiologically Based Kinetic (PBK) modeling, integrating known physiological, physiochemical and kinetic parameters with Quantitative Structure-Activity Relationship (QSAR) prediction and in vitro toxicity data describing hepatic metabolism. Results indicated that BEA tends to accumulate in adipose tissue of mice, while MON is partially excreted in rat and human urine. To address the yet undescribed occurrence of both toxins in humans, we aim to use *in vitro* bioassays and refine existing human PBK models to identify and quantify toxicological endpoints of concern for human health of BEA and MON. Different QSAR models were applied to predict potential toxicologic endpoints. Results were partially consistent with published data regarding the endocrine activity of BEA, but also predicted bioactivity that has so far not been assessed. Therefore, future research will focus on elucidating toxicity mechanisms for BEA and MON. Results of this research are expected to enhance global health and risk assessment for humans.

Keywords: mycotoxins, PBK modelling, QSAR, in vitro toxicity testing

Poster 07

Assessment of bisphenol A analogues for inhibition of the ovarian steroidogenic enzymes 3β-HSD2 and 17β-HSD1

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Endocrine-disrupting chemicals (EDCs) are exogenous substances or chemical mixtures affecting the hormonal system and causing adverse health effects. Given the rising rates of infertility in developed countries, a particular concern is the potential influence of EDCs on the female reproductive system. Female disorders such as polycystic ovary syndrome (PCOS) and endometriosis are endocrine-metabolic diseases likely aggravated by EDCs. Two key enzymes in ovarian steroidogenesis are 3β-hydroxysteroid dehydrogenase type 2 (3β-HSD2) and 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1). 3β-HSD2 catalyzes the biosynthesis of progesterone from pregnenolone, while 17β-HSD1 catalyzes the conversion of estrone to estradiol. Bisphenol A (BPA), a recognized EDC undergoing extensive investigation, has been found to inhibit 3β-HSD2 and 17β-HSD1. In contrast, newer BPA substitutes are less well characterized, emphasizing the need to assess their potential toxicities.

We investigated eleven BPA substitutes, namely BPAF, BPB, BPE, BPF, BPS, BPZ, BADGE, cyclo-di-BADGE, BPSIP, BPTMC, and TBBPA, and compared their potential to inhibit human 3β-HSD2 and 17β-HSD1 with that of BPA. We performed enzyme activity assays for human 3β-HSD2 and 17β-HSD1 using lysates of HEK-293 cells overexpressing the respective enzyme in the presence of vehicle or 10 µM of the aforementioned chemicals and determined the IC50 values for the positive hits.

We found that BPA (IC50 = 8 µM), BPAF (IC50 = 2.0 µM), BPB (IC50 = 1.9 µM), BPTMC (IC50 = 4.4 µM), and TBBPA (IC50 = 1.3 µM) inhibited 3β-HSD2 activity. Notably, the inhibition of 3β-HSD2 by these BPA substitutes was more pronounced than that by BPA. Moreover, 17β-HSD1 was inhibited by BPAF (IC50 = 16 µM) and TBBPA (IC50 = 0.74 µM), but not BPA.

In conclusion, our results show that four BPA replacement compounds inhibited human 3β-HSD2, and two inhibited 17β-HSD1, raising concerns about their safety as BPA substitutes. Moving forward, we plan to further investigate their impact of these chemicals on steroidogenesis by conducting activity assays for enzymes such as aromatase (CYP19A1) for a more comprehensive understanding. Additionally, we aim to assess the potential effects of these compounds on the expression of steroidogenic genes and measure steroid profiles in ovarian granulosa cells.

Poster 08

Painting the Fish Brain: Image-based Profiling of RTbrain Cells to Assess Neuroactive Chemical Toxicity

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Fish are pivotal in evaluating the environmental impact of chemicals, where the effects on survival and growth are commonly assessed in laboratory experiments as indicators of fish population health. The application of fish cell-based models using permanent fish cell lines offers a promising avenue to significantly reduce, and possibly eliminate, the need for animal testing. Notably, this progress extends to the promising field of replacing whole-animal experimentation. Despite its success and international standardization as a test guideline (ISO Standard 69933 and OECD Test No. 249) for fish cell-based testing, the acute cell toxicity assay using permanent fish gill cell lines from rainbow trout (Oncorhynchus mykiss, RTgill-W1 cell line) encounters limitations. Specifically, the assay fails to accurately predict toxicity for chemicals with reactive modes of action and neurotoxicants like allyl alcohol, lindane, permethrin, and caffeine. A potential solution lies in exploring the use of the RTbrain, a fish neuronal cell line, derived from the brain of rainbow trout, coupled with high-content, image-based assay used for morphological and phenotypical profiling by multiplexing fluorescent dyes. This novel approach could serve as a valuable animal-free tool for delving into the neurotoxic modes of action associated with environmental chemicals.

The according assay uses up to five fluorescent stains to mark major components of the RTbrain cells, namely the nucleus, mitochondria, lysosomes, cytoskeleton, and endoplasmic reticulum. Briefly, 24h after the cell seeding in a 96-well plate, cells are subjected to chemicals in a minimum of four concentrations in a time-resolved manner. The impact of chemicals is assessed during the lag phase, exponential phase, and stationary phase of cell population growth. The images are acquired using Agilent Cytation 5 using 40x high-contrast objectives. Nine fields of view are monitored per well to capture enough cells per chemical insult. Images are processed by segmenting cell organelles and various cell compartments (membrane, cytoplasm, and perinuclear space), followed by quantifying intensities, texture, shape, and morphology of each segment using Cell Profiler. Image-based cellular phenotype profiling will be used to assess the cellular health of chemically exposed cells in comparison to unexposed RT brain cells.

The approach shown here has the potential to serve as a robust source of data for further investigating chemical-induced alterations in fish cells. The phenotype of a targeted cell proves sensitive, with a subset of phenotypic features deviating from those of healthy cells, serving as a

distinctive fingerprint to characterise the early biochemical profile of cells undergoing chemical toxicity.

Poster 09

Gaps and needs analysis, barriers, opportunities and drivers for implementation of Artificial Intelligence and Machine Learning (AI/ML) tools in regulatory risk assessment

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Within the context of the Partnership for the Assessment of Risks from Chemicals, the present study aimed at identifying gaps and needs, barriers, opportunities, and drivers for the implementation of AI/ML technologies in chemical risk assessment (CRA) to facilitate further development of scientific criteria for the regulatory acceptance of AI/ML approaches.

Building on our prior review of regulatory and technological landscapes in toxicology and CRA for AI/ML application, this study focuses on three essential domains necessary for successful AI/ML integration into CRA: technology's interpretability and validity, organizational readiness and personnel competence, and the legal framework for technology use. Identified gaps and barriers in the technological domain include the scarcity of high-quality, standardized datasets vital for AI model training and validation, and the prevalent "black box" nature of deep learning algorithms, which obstructs interpretability and regulatory acceptance. Additionally, technical integration issues with existing CRA frameworks necessitate substantial adaptations. Organizationally, there exists a critical gap in requisite expertise among personnel, compounded by resistance to change and limited resources, underscoring the need for dedicated training programs and enhanced stakeholder collaboration. Legally, the existing regulatory landscape fails to fully encompass the nuances of AI, with gaps in guidance and standards, alongside concerns over intellectual property, data privacy, and the assignment of liability. The disparity in legal and regulatory stances on AI across jurisdictions further complicates global harmonization efforts. Main opportunities and drivers for integration of AI/ML tools from a regulatory science perspective lie in the ongoing transition to NAMs and NGRA, leveraging advancements in AI algorithms and computational power to improve toxicology predictions and risk assessments. Organizational drivers such as digital transformation and interdisciplinary training enhance CRA processes and decision-making. Legally, updating frameworks and fostering international harmonization facilitate AI adoption. Public engagement and a focus on sustainability further drive AI's role in CRA, promising more effective risk management and health protection.

In conclusion, the development of scientific criteria for regulatory acceptance of AI/ML approaches should take into account identified gaps and needs. Furthermore, it should be tailored to identified domains of possible AI/ML tools application, namely: (i) Data/evidence management tools; (ii) Data/evidence generation tools - mainly modelling tools helping to create new types of data within hazard and exposure assessment; (iii) Decision support tools that help integrate all the data and make conclusions. Criteria may also take advantage from already existing frameworks for assessment of computational tools in toxicology, e.g. OECD (Q)SAR Assessment Framework.

Poster 10

New Approach Methods for Hazard Assessments of Medical Devices Constituents- A Case Study of Phenetole -

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Medical devices are required to be free from unacceptable toxicological risks during clinical application. Manufacturers shall conduct a toxicological risk assessment including hazard assessment for all components of the medical device according to ISO 10993-17:2023. The standard allows to apply new approach methodologies (NAMs), such as *in silico* modeling and read-across techniques, as well as the threshold of toxicological concern. In line with 3R, this allows a reduction of animal testing for various toxicity endpoints (i.e. irritation, sensitization, systemic toxicity, genotoxicity, reproductive toxicity, carcinogenicity).

Here, we present a case study applying the new ISO 10993-17:2023 methodology to the data-poor Phenetole present on an implant. For the hazard assessment we combined an in-silico analysis (i.e. Toxtree, Danish QSAR, QSAR Toolbox) with a read-across grouping approach to evaluate several toxicological endpoints, such as toxicokinetics, irritation, sensitization, systemic effects, reproductive and developmental toxicity, genotoxicity, and carcinogenicity. This adapted strategy, based on a previously published scientific contribution by C. Laupheimer, S. Parween, Y. Kolianchuk and A. Jaksch ("New approach methods for challenging toxicological risk assessments of medical devices. A case study of phenyl propyl carbonate", ESTIV 2024, Poster 40) enabled us to conduct a hazard assessment of a data-poor substance, thereby eliminating the need for further testing and reducing the number of *in vivo* experiments. Phenetole was found to be a Cramer class I substance. This case study highlights the utility of NAMs in addressing data gaps concerning toxicological risk assessment of medical devices.

Poster 11

Linking Transcriptome in Endocrine Disrupter-Exposed Developing Rat Hippocampus with Impaired Memory Function in Adult Offspring and Human Stem Cell-Derived *In Vitro* Models

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To develop novel approaches of developmental neurotoxicity testing, effects of chemicals on OMICs in developing rat hippocampus were investigated as a link between *in vivo* and *in vitro* data. The chemicals were associated with impaired children's behavior in the SELMA study: Bisphenol F (BPF, 3.6 or 0.036 mg/kg), butylbenzylphthalate (BBzP, 200 or 20 mg/kg), cyclohexane dicarboxylic acid diisononyl ester (DINCH, 300 or 30 mg/kg), perfluorooctanesulfonic acid (PFOS, 0.75 or 0.3 mg/kg) permethrin (PMT, 3.6 or 0.36 mg/kg) or triphenylphosphate (TPHP, 20 or 2 mg/kg) was administered in chow to F0 rat dams from pre-mating until lactation. The higher dose was chosen from reprotox data. Hippocampus was taken on postnatal day 6 (PND 6) for transcriptomics, epigenomics and metabolomics in the same tissue sample. One pup/sex/litter of the higher dose group was raised to adulthood to test memory and other behaviors.

Memory function (Morris water maze) was impaired by BPF and BBzP in adult male and by DINCH and TPHP in adult female offspring. PFOS was ineffective, PMT could not be tested. Transcriptome of PND 6 hippocampus was correlated with behavior in adult offspring. Weighted gene co-expression network analysis (WGCNA) identified gene modules in PND 6 hippocampus significantly associated with chemical treatment and escape latency in Morris water maze in a sexually dimorphic manner. Genes linked with impaired memory function included proneural factors, factors inhibiting neuronal differentiation, Wnt and Notch signalling, genes involved in cell cycle regulation, differentiation, synaptogenesis and epigenetics.

Interneuron subtype specification was affected in medial and caudal ganglionic eminence-derived interneurons. Comparisons of transcriptome of human stem cell-derived brain organoids and PND 6 hippocampus suggest that it may be possible to characterize treatment effects on corresponding functional modules according to sexual phenotype and genotype.

Our study revealed gene expression patterns induced by environmental chemicals in developing hippocampus that are linked with behavioral outcome. The combination of transcriptomics, epigenetics and metabolomics is expected to identify molecular features that could be tested for their predictive value.

Poster 12

An Integrative Framework for the Identification and Ranking of Endocrine Disrupting Chemicals (EDCs)

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Endocrine-disrupting chemicals (EDCs) are a global public health challenge due to their ubiquity, complexity and associated adverse health effects. EDC exposure induces metabolic and neurodevelopmental disorders and is widely associated with the reduction of fertility rates in developing countries. Despite EDCs being a priority for regulatory agencies, only a small fraction of chemicals authorized for commercial use in the European market have been characterized for ED-activity. To streamline this process, in silico methods are becoming fast and reliable tools to infer chemical toxicity.

The aim of this project is to generate and validate a machine learning framework to identify and rank chemicals according to their potential ED-activity. To do so, we first (1) generated an integrative database containing physicochemical, human toxicological, and ecotoxicological properties of more than 100 000 EU-relevant chemicals. The database includes over 60 000 parameters, including partition coefficients, chemical-gene/protein associations, hormone binding assays, ecotoxicity for aquatic species and similarity to structural alerts related to endocrine disruption, among others. Secondly, (2) we developed a web-based exploratory tool that allows users to explore chemicals, their properties, and structural similarities. Thirdly, (3) we implemented a machine learning model to identify and rank chemicals based on their potential ED-activity. To improve the transparency of the model, SHAP was used to define chemical properties contributing the most to each prediction. Finally, (4) the top-ranking unknown EDCs will be further validated using *in vitro* reporter assays.

In conclusion, we are developing a unique and integrative open-source resource for scientists and regulators to evaluate and identify novel EDCs. Our outcomes can be used to prioritize the risk assessment of EDCs, thereby promoting a shift in Swiss and EU chemical regulations towards a toxic-free environment.

Poster 13 (selected for Short Oral Presentation)

Real-time chemical toxicity and water quality monitoring with fish cell lines

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In ecotoxicology, fish toxicity testing is a crucial component of chemical risk assessment and effluent testing, and there is a growing need for alternative methods to reduce the use of live animals. Fish cell lines can be used as an effective *in vitro* alternative to predict adverse outcomes, including the most commonly tested one: acute toxicity. However, most *in vitro* assays using cell lines are carried out under static exposure conditions and assess cell viability only at a single time point, after chemical exposure. Thus, no information is gained on how toxicity develops over time. In this work, we established a microfluidic system for acute toxicity testing under flow conditions in real-time. We employ Electric Cell-substrate Impedance Sensing (ECIS), a label-free and non-invasive technique, which allows continuous monitoring of cell viability during chemical exposure. We use cell lines from the rainbow trout (*Oncorhynchus mykiss*), a standard species in ecotoxicity testing, of which there are several established cell lines derived from various organs. Using a cell line from the gill, RTgill-W15, we demonstrate the application of this method with three model compounds of different physico-chemical properties and toxic modes of action. Our results show that different chemicals elicit different toxicity profiles over time. In addition, permanent and reversible changes can be distinguished during a depuration phase after exposure. To investigate chemical impacts across multiple organ systems, we are currently expanding the system to incorporate cell lines from the intestine, liver, and brain of rainbow trout. This platform has application for time- and concentration dependent chemical toxicity screening as well as the evaluation of water samples. In addition, we are working on an adaptation of this system into a portable field instrument. This includes fully automated continuous sampling of water, sample preparation, and exposure of cells, with on-line availability of data in real-time. This will allow monitoring the effects of spatially and temporally varying mixtures of chemicals as they occur in the aquatic environment.

Keywords: aquatic ecotoxicology, alternatives to animal testing, fish cell lines, impedance sensing, ECIS, lab-on-chip, environmental monitoring

Poster 14

Mitochondrial function in an *in vitro* model of Familial Alzheimer's disease

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Alzheimer's disease (AD) is a neurodegenerative disorder marked by amyloid beta (Aβ) plaques, neurofibrillary tangles, and neuroinflammation, leading to neuronal and synaptic loss. Mitochondrial toxicity is a crucial factor in AD progression, impairing energy production, increasing oxida-tive stress, and exacerbating neuronal dysfunction and degeneration. This study investigates mitochondrial toxicity in neurons and astrocytes with familial Alzheimer's disease (FAD) mutations to identify specific mitochondrial deficits driving neurodegeneration and axonal dysfunction.

We modified ReN-VM neural progenitor cells to express FAD mutations in APP (ReN-GFP-APP) alone or with mutated PSEN1 (ReN-GFP-APP-PSEN). Neuro-astroglial morphology and the deposition of toxic Aβ and p-Tau were analysed via immunofluorescence and ELISA. Mitochondrial distribution and neuronal morphology were examined via confocal microscopy while membrane potential was assessed using TMRE staining, providing insights into mitochondrial activity and stability. Additionally, superoxide (O_2^-) production was quantified using Mitosox dye to evaluate oxidative stress and mitochondrial dysfunction.

ReN-GFP-APP and ReN-GFP-APP-PSEN cells differentiated into neurons and astrocytes. ReN-GFP-APP-PSEN cells showed increased Aβ deposition and p-Tau after 6 and 9 weeks of differentiation, respectively, leading to create a toxic environment and mitochondrial dysfunction. Compromised mitochondrial membrane potential and state of oxidative stress was shown by a reduction in TMRE-positive mitochondria and elevated ROS levels in ReN-GFP-APP cells at 6 weeks. ReN-GFP-APP-PSEN cells showed increased mitochondrial fission and reduced number of mitochondria, demonstrated by TOMM20 staining, suggesting impaired mitochondrial dynamics and potential mitophagy. Additionally, Tuj1-positive FAD neurons showed significant axonal degeneration, indicating neurotoxic effects.

Overall, our results demonstrate a strong link between mitochondrial dysfunction, oxidative stress, and neuronal damage in an FAD *in vitro* model, suggesting that exposure to mitochondrial toxicants may exacerbate AD-related neuronal damage. Understanding these toxicity pathways is crucial for developing targeted therapeutic interventions.

Poster 15

Unanticipated differences in the rat plasma metabolome of Genistein and Daidzein

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Genistein (GEN) and daidzein (DAI) are soy isoflavones known for their interaction with oestrogen receptors, exhibiting both health benefits and concerns in humans. In this study, we conducted 28-day oral studies in male and female Wistar rats to elucidate (1) changes in metabolites, (2) compare their metabolomes with other compounds, and (3) identify toxicological modes of action (MoA).

Dose levels for GEN were 1000 and 300 mg/kg bw by gavage and 1000 and 300 ppm (via diet). DAI gavage dose levels were 1000 and 100 mg/kg bw. Results were evaluated using the MetaMap®Tox data base. Both compounds demonstrated metabolome profiles associated with estrogenic profiles and compounds, predominantly in females. However, the metabolomes were compound-specific with relatively few common metabolite changes. Notably, there were no relevant matches between any GEN and any DAI treatment group indicating that both compounds are substantially different from metabolome perspective.

Ranking of the metabolome patterns for GEN and DAI with ≥ 1000 compounds in the MetaMap®Tox database revealed correlations with estrogenic and other hormonally active compounds. Furthermore, the metabolome of GEN-treated females correlated best with Cabergoline, a dopamine D2 receptor agonist, while DAI females with tamoxifen and diethylstilbestrol, suggesting that even their estrogenic activity may be different. Beyond estrogenic effects, the high dose (HD) DAI metabolome indicated altered fatty acid metabolism associated with PPAR-alpha activation, while GEN showed indications of ethanolamine-like liver effects. Dose levels without estrogenic effects for GEN were 1000 and 100 mg/kg bw for males and females respectively, there were no estrogenic effects in the feeding studies. For DAI, the no estrogenic effect level was 300 mg/kg bw for males and <100 mg/kg bw for females, suggesting that DAI may be a more potent oestrogen than GEN in rats.

Keywords: Metabolomics, Genistein, Daidzein, Estrogens

Poster 16

Human gut microbiota-mediated metabolism of the chloroacetamide herbicide metazachlor

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Chloroacetamides are globally widely used herbicides, and metazachlor, as a representative of this class, is known for its environmental persistence and hydrolytic stability. While metazachlor undergoes biotransformation in soil and aquatic microbiota, its metabolism by the human gut microbiota and the potential toxicological implications remain unexplored. This study aimed to characterize how metazachlor may be metabolized by human gut microbiota and assess potential toxicological risks presented by metabolism products. Therefore, we exposed human gut microbial communities derived from human fecal samples to metazachlor at levels equivalent to, as well as three times higher or lower than, the EFSA-derived acceptable daily intake level. Using untargeted LC-MS/MS analysis, we identified 6 known and 18 previously unreported metabolites of metazachlor, which were organized into proposed biotransformation pathways. Overall, the metabolic processes in the gut microbiota, which are dominated by altered sulfide chemistry, differ greatly from those in soil and aquatic microbiota, which involve the formation of corresponding oxanilic acid and sulfonic acid metabolites. Moreover, it was shown that the metabolite arising from the reaction between cysteine and metazachlor is formed non-enzymatically, which is different from soil and aquatic microbiota metabolism where the major metabolism is thought to originate from glutathione conjugation. To further support our proposed metabolic pathways, we synthesized several key intermediate metabolites to repeat the fermentation experiment with them and to track formation of downstream metabolites. To assess toxicological relevance of identified metabolites of metazachlor, QSAR predictions using OCHEM web platform were used to predict the likelihood that newly identified metabolites possess activity toward targets of interest, particularly CYP inhibition, endocrine disruption, hERG inhibition, and other responses. Notably, up to 30% of metazachlor is converted into the disulfide of metazachlor thiol, which, according to QSAR predictions, may act as a modulator of the widest variety of biological targets among all identified metabolites. Further research will involve experimental testing by in vitro toxicity assays relevant to the predicted toxicological endpoints. Our findings reveal that metazachlor is extensively metabolized by the human gut microbiota, with several newly identified metabolites potentially posing toxicological risks, paving the way for further research into their effects on human health.

Keywords: metazachlor, gut microbiota, metabolism, LC-MS/MS, QSAR

Poster 17

Assessing gut microbiota's role in phytoestrogen bioactivation

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Isoxanthohumol (iXN), a prenylated polyphenol found naturally in hops and dietary supplements for the relief of postmenopausal symptoms, is biotransformed via O-demethylation by the gut microbiome to 8-prenylnaringenin (8-PN), the most potent phytoestrogen known, which interacts with estrogen receptors and may disrupt endocrine signaling. The potential adverse effects of 8-PN are poorly understood due to the difficulty in determining its systemic and tissue concentrations. To address this, we developed a human-specific physiologically based kinetic (PBK) model designed to predict the biologically available levels of hop-derived polyphenols and their microbial metabolites in human blood and target tissues based on realistic exposure scenarios. We integrated metabolic rates obtained from glucuronidation assays using hepatic and intestinal S9 fractions and the kinetic rate of microbial metabolism in anaerobic fecal fermentation. The conversion of iXN to 8-PN was quantified using liquid chromatography-mass spectrometry and these values were used to derive iXN metabolism. These data were used to establish a new kinetic model incorporating the gut microbiome as a distinct metabolic compartment, allowing the assessment of inter-individual variability in 8-PN formation depending on microbiome composition. We then performed *in vitro* cellular assays to evaluate estrogenic activity and further assess the safety of hop polyphenols. The outcomes advance our understanding of the toxicokinetics of phytoestrogens and provide a robust framework for the quantitative assessment of gut microbial metabolites in chemical safety assessment.

Keywords: biotransformation, microbial metabolism, pharmacokinetics, PBPK modeling, hop polyphenols, estrogenicity

Poster 18 (selected for Short Oral Presentation)

Click-chemistry-aided quantitation and sequencing of oxidized guanines and apurinic sites uncovers their transcription-linked strand bias in human cells

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DNA modifications drive aging, neurodegeneration, carcinogenesis, and chemotherapy drug action. To understand the functional genomic roles of DNA modifications, it is critical to accurately map their diverse chemical forms with single-nucleotide precision in complex genomes, but it remains challenging. Click-code-seq is a click-chemistry-aided single-nucleotide-resolution strategy for guanine-oxidation mapping, used in yeast DNA but having poor applicability to human genomes. Here, we upgraded click-code-seq to enable its first application for sequencing DNA oxidation and depurination in human genomes. For this, we developed a companion fluorescence assay, click-fluoro-quant, to rapidly quantify different common DNA modifications, and devised novel adapters to minimize false modification detection and assess modification frequency in cell populations. We uncovered that endogenous DNA oxidation in a human cell line has a highly similar pattern to cancer mutational signatures associated with reactive oxygen species. We established that the DNA-alkylating chemotherapy drug irofulven preferentially induces depurination in ApA dimers and promoter regions. Intriguingly, we revealed that oxidized guanines and apurinic sites, both irofulven-induced and endogenous, are depleted in gene transcribed strands, and the strand bias widens with increasing gene expression. This work substantially advances click-code-seq for deciphering the impacts of key modifications in human DNA on cellular physiology and toxicological responses.

Poster 19 (Late submission)

ToxOligo – Interference of oligomers released from food contact materials with steroid hormone action

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Most food packaging materials contain non-intentionally added substances (NIAS) such as oligomers derived from polymerisation of plastic materials. These compounds can migrate from the packaging material into food, exposing consumers orally. The ToxOligo project aims to fill a knowledge gap by analyzing biological activities of oligomers. The project mainly focuses on the influence of oligomers on the activities of steroid metabolizing enzymes, steroid receptors and steroid-responsive immune cells.

A series of oligomers were screened for potential inhibitory effects on 11β-HSD1 and 11β-HSD2 that interconvert active and inactive glucocorticoids as well as 17β-HSD3 that catalyzes the last step of testosterone biosynthesis. Additionally, transactivation experiments were conducted to evaluate the potential agonistic and antagonistic activities of the selected compounds on AR, PR, GR, MR, ERα, and ERβ. Moreover, all oligomers were tested for their effects on the synthesis of pro-inflammatory cytokine in THP-1 cells stimulated with 0.1 or 1 ng/mL LPS.

Except for perfluorooctanoic acid, which reduced 11β-HSD1 activity at a concentration of 10 µM, the radioactivity assay showed no enzyme inhibition by the tested compounds. With respect to the steroid receptors, ethylene sebacate showed GR agonistic activity. Another molecule, phthalic acid cyclic hexamethylene ester, had weak ERα agonistic activity. At 0.1 ng/ml LPS, none of the oligomers showed a pro-inflammatory response. However, linear compounds like glycols, terephthalic acid derivatives and cyclic compounds reduced the immunological response to 1 ng/ml LPS, suggesting a compromised immune response.

Our understanding of the potential effects of oligomers on human health is limited. ToxOligo contributes to fill this knowledge gap. Further research is needed to assess the safety of oligomers for consumers.

Keywords: oligomers, *in vitro* tests, steroid hormone action