Annual Meeting 2025 – Poster Session

Poster 01

Developing mechanistic and responsive biomarkers of aging based on genome-wide maps of DNA breaks and oxidation

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There is a lack of biomarkers reflecting molecular processes that mechanistically determine aging phenotypes. DNA damage is a primary hallmark of aging and contributes to cellular dysfunction, yet its genome-wide dynamics and functional and molecular consequences remain poorly understood. Abundant forms of DNA damage are DNA breaks and oxidation, such as 8-oxoguanine. Emerging data based on single-nucleotide resolution 8-oxoguanine maps suggests a link between DNA oxidation and methylation as well as a coupling of oxidative damage repair and transcription.

To create mechanistic biomarkers of aging, we employed novel sequencing methods, click-code-seq and GLOE-seq, to identify genome-wide profiles of abundant and relevant forms of DNA breaks and oxidation during aging. Livers taken throughout the healthy mouse lifespan were sequenced to profile DNA-damage changes that occur in aging. To assess the responsiveness to accelerated and decelerated aging, the DNA-damage biomarkers were tested in mouse cohorts that receive genetic or dietary interventions. For all mouse genomes, we also profiled RNA-seq, ATAC-seq and DNA methylation as a basis for evaluating functional implications of DNA-damage levels in aging and lifespan-modulating interventions.

We aim to test how DNA methylation clocks may be explained by DNA-damage patterns, revealing genomic mechanisms of aging embedded in key processes of DNA damage, repair, and epigenetic regulation.

Keywords:

Poster 02

Developing an *in vitro* fish cell-based model for environmental neurotoxicity testing

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Chemicals released into water bodies pose a major environmental problem. Among other impacts, they can affect the neurophysiology of aquatic organisms, compromising their survival. Traditional environmental risk assessment relies on time- and resource-intensive animal experiments - alternative methods such as those based on permanent fish cell lines are in urgent demand. However, detecting tissue-specific toxicity, such as neurotoxicity, remains challenging and limits the regulatory acceptance of animal-free approaches.

Here, we propose the rainbow trout (Oncorhynchus mykiss) brain cell line (RTbrain) as an *in vitro* model for neurotoxicity testing of environmental pollutants. The project aims to (i) characterize RTbrain cells and (ii) develop an RTbrain-based *in vitro* bioassay for screening neurotoxic chemicals.

Baseline transcriptomic profiling revealed enrichment of genes related to central nervous system development, neuronal differentiation, and neuroactive ligand-receptor interactions, suggesting the presence of neuronal progenitor and mature neuronal features. Gene expression and immunocytochemistry confirmed the expression of markers for neuronal progenitors, astrocytes, oligodendrocytes, and mature neurons. Transcripts involved in myelin sheath formation further support the presence of oligodendrocyte-like cells and overall culture heterogeneity. Preliminary electrophysiological data showed weak and unsynchronized activity, consistent with a mixed cell population.

For the RTbrain-based bioassay, a positive control for general cytotoxicity was established based on OECD TG249, using sodium dodecyl sulfate (SDS) and three complementary viability assays assessing metabolic activity, cell membrane integrity, and lysosomal membrane stability. Cytotoxicity data for reference neurotoxicants are currently being generated. Subsequent analyses will target sublethal effects, including oxidative

stress, mitochondrial dysfunction, and changes in selected marker genes associated with neurotoxicity.

Keywords:

Poster 03 (selected for Short Oral Presentation)

Challenging the standard use of male physiology in next-generation risk assessment: Sex-informed physiologically based kinetic modelling

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To enhance the relevance of next-generation risk assessment (NGRA) and promote its adoption over traditional animal-based approaches, it is essential to consider the physiology of the entire human population. Physiologically based kinetic (PBK) modeling provides a framework to simulate xenobiotic absorption, distribution, metabolism, and excretion (ADME) by integrating physicochemical properties with human biological data and diverse experimental sources. Currently, PBK models often neglect sex-specific differences, limiting their applicability largely to male physiology. This gap can lead to inaccurate ADME predictions for women and increases uncertainty for risk assessment, particularly when tissues and outcomes particularly relevant to female health are of toxicological concern.

Here, we present a PBK modeling strategy that explicitly incorporates both male and female physiology through sex-specific parametrization of body composition, enzyme expression, and biodynamic processes. The sex-informed models were evaluated against human pharmacokinetic data for documented sex-dependent adverse drug reactions, accurately predicting 81% of measured sex-specific outcomes. Furthermore, this framework aims to extend the predictive scope of new approach methodologies (NAMs) by incorporating key female-specific compartments (breasts, uterus, and ovaries) using detailed anatomical and histological data. Thereby, this work advances *in silico* modeling for female-specific conditions and for understanding adverse outcomes linked to menstrual cycle, pregnancy, menopause, and certain gynecological diseases.

Keywords: pharmacokinetics, sex differences, NGRA, female health

Poster 04

From DNT to DART: leveraging a NAM success story

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Regulatory hazard and risk assessment of chemicals is shifting from animal apical endpoints to human-relevant new approach methodologies (NAMs). Traditional animal studies are time- and resource-intensive, ethically problematic, and limited by interspecies differences. A domain of particularly high animal use and pronounced species specificity is developmental and reproductive toxicity (DART). Although exposures to certain chemicals have been associated with infertility, miscarriage, and birth defects, human-relevant test systems are limited, while the need to characterize DART profiles for a large number of chemicals is substantial.

The International STakeholder NETwork (ISTNET)-DART aims to build consensus on the development and use of *in silico* and *in vitro* methods to define a NAM-based integrated testing strategy that meets regulatory requirements for DART. The approach follows the successful ISTNET initiative for developmental neurotoxicity (DNT), where early involvement and dialogue among regulators, industry, and academia culminated in OECD Guidance Document No. 377. The first ISTNET-DART meeting (12–13 September 2024) followed this strategy and was intended to develop a roadmap for NAM-based DART testing involving all stakeholders.

The meeting brought together 61 participants from 12 countries across regulatory, academic, and industry sectors. It opened by outlining the DNT pathway. For DART, 18 short talks covered reproductive and developmental biology, the status of *in vitro* methods, and physiology-based kinetic modelling. The talks were framed by regulator perspectives on current DART strategies, their perspectives on the use of NAMs in this complex area, their integration into next generation risk assessment frameworks, and views on readiness and validation. Consensus points included the challenge to cover all DART life-stages, the need for DART *in vitro* batteries and tiered strategies, limited AOP coverage, and the value of *in silico* tools. Next steps are to disseminate outcomes as a DART roadmap to engage more scientists and regulators.

By replicating the ISTNET model with early stakeholder alignment and a biology-first assay design, ISTNET-DART aims to support efficient tiered DART testing that delivers comprehensive, regulatory-relevant human health assessment.

Keywords: developmental toxicity, reproductive toxicity, New Approach Methodologies

Poster 05

Nanoplastic Uptake and Barrier-Related Responses in Human Intestinal and Brain Endothelial Models

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Nanoplastics (NPs) are emerging environmental contaminants known to enter the human body through ingestion or inhalation and subsequently reach the systemic circulation. The aim of this study was to investigate how nanoplastic exposure affects the integrity of epithelial and endothelial barriers, particularly intestinal and brain vascular (BBB) models.

To this end, human cerebral microvascular endothelial cells (hCMEC/D3), intestinal epithelial cells (Caco-2), and human iPSC-derived gut organoids were used. Cytotoxicity was evaluated using the CCK-8 assay. After nanoparticle treatment and incubation, cells were fixed and immunostained for LAMP-1 and tight junction proteins (ZO-1) to visualize NP lysosomal uptake and junctional organization by confocal microscopy. Barrier integrity was further examined by measuring transepithelial electrical resistance (TEER) and Lucifer Yellow permeability. In addition, NP uptake into gut organoid cells was analyzed following microinjection into the lumen.

The results showed that microplastic exposure did not markedly affect cell viability but led to intracellular accumulation of NPs. Notably, in Caco-2 cells, NP uptake was size-dependent and the internalized particles showed lysosomal localization (LAMP-1-positive vesicles). In contrast, NPs of all tested sizes were taken up by the endothelial cells (hCMEC/D3). TEER measurements in Caco-2 monolayers revealed no significant change in barrier electrical resistance following NP exposure. However, subtle junctional alterations were observed by ZO-1 staining, and Lucifer Yellow permeability suggested a trend toward mildly increased paracellular leakage.

In conclusion, these findings indicate that nanoplastics can be internalized by both endothelial and epithelial barriers and may induce early or subtle perturbations in barrier function underscoring the potential risk for translocation of foreign particles and initiation of systemic or inflammatory processes.

Keywords:

Poster 06

Mechanical Properties of HepaRG Spheroids in the context of Liver Fibrosis

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Liver fibrosis is a prevalent global disease and a converging point for chronic liver damage. It is a progressive condition characterized by excessive extracellular matrix (ECM) deposition and increased stiffness. This study aimed to investigate the nanomechanical properties of HepaRG spheroids, and their response to the profibrotic cytokine Transforming Growth Factor-β1 (TGF-β1) using Atomic Force Microscope (AFM). In parallel, we assessed changes in ECM synthesis and deposition to link molecular alterations to nanomechanical phenotype.

HepaRG spheroids (12K and 14K cells/spheroid) were generated to reproduce a liver-like architecture. Spheroids were treated with 1 ng/ml of TGF-β1 for 48 hours, a commonly used model mimicking fibrogenic signaling during chronic chemical-induced liver injury. Nanomechanical characterization of control and treated spheroids was performed using the ARTIDIS® AFM platform. RT-qPCR and immunofluorescence (IF) staining were used to quantify ECM-related gene and protein expression.

TGF-β1 treated spheroids (N = 26) exhibited clear stiffening compared with controls (N = 34). The stiffening was primarily attributable to enhanced ECM deposition, as the cellular component showed no significant differences in the nanomechanical properties. Gene expression analysis revealed an upregulation of COL1A1, while fibronectin (FN1) transcript levels remained stable. IF confirmed increased FN1 accumulation within the spheroid matrix.

AFM-based nanomechanical phenotyping clearly distinguishes TGF-β1-treated and control spheroids, correlating with increased ECM deposition and collagen expression.

This demonstrates the value of AFM as a biophysical marker for detecting early fibrotic remodeling in 3D liver models. The next phase will involve 3D HepaRG–hepatic spheroids that include hepatic stellate cells, main sources of ECM, to investigate the multicellular dynamics of liver fibrosis

Keyword: Liver fibrosis, Extracellular matrix, HepaRG spheroids, Atomic Force Microscopy, nanomechanics, ARTIDIS ART-1

Poster 07

Establishment of Readiness Criteria for AI-based tools for Risk Assessment: READYAI

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Recent advances in artificial intelligence (AI), machine learning (ML), and deep learning (DL) are leading to a new era of data-driven innovation, significantly impacting the field of chemical risk assessment. These advanced computational techniques are increasingly being recognized as powerful tools for various applications in toxicology, including data retrieval (e.g. systematic reviews), predictive modeling (e.g. toxicokinetics, quantitative structure-activity relationships (QSARs), read-across), toxicity prediction, and evidence integration. To effectively and reliably integrate these tools into regulatory frameworks, it is essential that regulatory authorities, policymakers, and other stakeholders gain a deeper understanding of their nature, applicability, validity, and inherent limitations. It is equally crucial for computational scientists and AI developers to understand regulatory requirements and prerequisites. To maximize the benefits of AI in chemical safety assessment, a skilled and multidisciplinary task force is needed, fostering collaboration among regulators, toxicologists, computational scientists, and AI developers for responsible utilization of AI-driven methodologies. Within this framework, and as part of the Partnership for the Assessment of Risks from Chemicals (PARC) initiative, we have developed the project READYAI. We aim to address the key challenges associated with implementing AI in toxicology by developing a comprehensive scoring system for evaluating the development, training, and validation of AI-based tools applied in chemical risk assessment, enabling a more independent and objective assessment of the regulatory applicability of AI/ML/DL-based tools. Key concerns include ensuring the reliability of AI-generated predictions, mitigating biases in training data, improving algorithm interpretability, explainability, reproducibility & transparency, and addressing legal and ethical implications. By defining readiness criteria and adhering to best practice principles, READYAI seeks to facilitate regulatory acceptance of AI/ML/DL tools applied in chemical risk assessment.

Keywords:

Poster 08

A combined *in vitro* and *in silico* strategy to predict toxicokinetics of emerging mycotoxins

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Many mycotoxins that can be produced by fungi and contaminate food and feed are neither regulated nor routinely monitored in food, often due to limited available data concerning their toxicity. These are referred to as emerging mycotoxins and may negatively impact public health. To enhance our understanding of their mode of action and adverse health effects, this study endeavors to predict the toxicokinetics of selected emerging mycotoxins produced by Alternaria and Fusarium spp., aiming to predict internal exposure concentrations at organs of toxicological concern.

To pursue this goal, Physiologically Based Kinetic (PBK) models as an alternative method to animal experimentation were developed based on a recently established model concept. While physiological parameters could be extracted from literature, quantitative structure-activity relationship (QSAR) tools were employed to predict tissue partitioning and gastrointestinal uptake. Kinetics of phase I and II hepatic metabolism were assessed *in vitro* by following emerging mycotoxin biotransformation in incubations with rodent and human liver S9 and microsomal fractions, using LC-MS/MS analysis. Therewith, rodent PBK models for selected emerging mycotoxins were parameterized and their predictive accuracy was evaluated against already available toxicokinetic data from animal studies. Then, we performed cross-species extrapolation to develop human PBK models and used those to estimate mycotoxin concentrations in organs of toxicological relevance.

These developed quantitative and robust tools will be used for *in vitro* to *in vivo* extrapolation to support next generation risk assessment of emerging mycotoxins.

Keywords: mycotoxin, PBK modelling, QSAR, *in vitro* toxicity testing, toxicokinetic

Poster 09

Time-resolved LC–MS annotation of gut microbiome-mediated xenobiotic biotransformations

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Microbial biotransformation of chemicals shapes drug efficacy, safety, and leads to inter-individual variability in response. Yet, identifying microbial metabolites in complex biological matrices remains a major analytical challenge. Current untargeted LC–MS annotation workflows work well for known metabolites but dataset complexity, transient intermediates, and the absence of reference spectra for previously undescribed compounds present difficulties. To address this, we developed a computational pipeline integrating *in silico* biotransformation predictions using existing tools (Pickaxe, MicrobeRX) with preprocessed time-resolved LC–MS data. Detected features were annotated by mass matching to predicted metabolites, filtered against negative controls, and further curated through visual inspection of time-course trends to ensure biologically consistent formation patterns. We applied this approach to identify metabolites resulting from the degradation of fluorinated pharmaceuticals by human fecal microbiomes. We gained evidence for several previously unreported microbial metabolites. We confirmed the identity of a key microbial flutamide metabolite that was also previously attributed to hepatic metabolism, illustrating potential host–microbiome metabolism overlaps. Thus, we present a tool to integrate predicted microbial biotransformations with untargeted metabolomics data and applied it to identify microbial metabolites of fluorinated chemicals. This approach is expected to support future studies of microbiome-associated xenobiotic metabolism, and its implications for drug metabolism and safety.

Keywords: Metabolite annotation, Gut microbial metabolism, LC–MS, Biotransformation

Poster 10

Quantifying rate and global profile of gut microbiota metabolism ex vivo by LC-MS/MS

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The microbiota performs many chemical reactions within our gut with direct impact on human health. Sequencing of microbiota communities is insufficient to accurately decipher the chemical reactions occurring within the gut environment and which drive chemical toxicity. Thus, we use anaerobic fermentations of fecal microbiota, time-series sampling, and a merged targeted/untargeted LC-MS/MS analysis to quantify chemical transformation rates of known food, drug, and endogenous host metabolites. Metabolic profiles encompassing deglycation, nitroreduction, sulfoxide reduction, deglucuronidation, bile acid metabolism, benzisoxazole ring reduction, and unique food chemical transformations were generated for five human donors. Chemical reaction rates varied dramatically by chemical structure and across donors. Conditions of fermentation were tested including multiple growth media, fresh and frozen samples, and fecal slurry dilutions to determine an optimized protocol for *in vitro* cultivation of gut microbe communities. As expected, dilution of the inoculated microbiota influenced chemical reaction rates that are dependent on microbial metabolism (nitroreduction, deglucuronidation, etc.), but not reactions primarily driven by spontaneous reactions (chemical-matrix interactions). Fresh compared to frozen microbiota samples did not significantly change chemical reaction rates for targeted chemicals. Untargeted metabolomics analysis provided global insight into metabolic behavior of more than 300 annotated chemicals. *Ex vivo* fermentations monitored by LC-MS/MS provide quantitative metabolic profiles and global metabolism measurements applicable for individualized assessment of microbiota function that are not possible to glean from other -omics technologies. Our approach provides a basis to characterize chemical metabolism in the gut to more comprehensively assess and predict toxicity of drug, food, and xenobiotic chemicals.

Keywords: Gut microbiota, Metabolism, Mass spectrometry

Poster 11

Applicability of fish cell lines for the bioaccumulation and toxicity assessment of structurally diverse zwitterionic surfactants

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The environmental risk assessment of chemicals with complex physicochemical properties—such as those with both positive and negative charges, low solubility, and high hydrophobicity—poses substantial challenges for establishing accurate exposure conditions in experimental systems using fish. Zwitterionic surfactants, widely used in consumer products and frequently detected in aquatic environments, exemplify these difficulties. To address the lack of available data regarding their environmental risks and improve the mechanistic understanding of surfactant-organism interactions, we evaluated the applicability of animal-free testing methods, namely the rainbow trout (Oncorhynchus mykiss) cell lines RTgill-W1 and RTL-W1, to characterize the acute toxicity and bioaccumulation of structurally diverse zwitterionic surfactants differing in carbon-chain length and functional groups. Cell viability assays, following the OECD 249 test guideline, revealed that amine oxide congeners with 10-, 12-, and 14-carbon chains exhibited increasing toxicity with chain length (EC50 values: 138, 15, and 4 mg L-1, respectively), whereas their bioaccumulation potential showed the opposite pattern. In contrast, 16- and 18-carbon congeners showed no further increase in toxicity nor bioaccumulation, suggesting limited bioavailability due to their complex behavior in test media. Surfactants containing additional amide and carboxylate functionalities, such as cocamidopropyl betaine, demonstrated significantly high acute toxicity (EC_{50}: 5 mg L^{-1}) and the highest bioaccumulation among all tested compounds (BCFgill: 303; BCFliver: 264). Despite these findings, all tested surfactants remained below regulatory bioaccumulation thresholds under REACH. Collectively, these results reinforce the relevance of fish cell line–based assays as robust, animal-free tools to support the environmental risk assessment of structurally diverse zwitterionic surfactants.

Keywords: Surfactants, Bioaccumulation, Toxicity, Fish cell lines, Risk assessment

Poster 12 (selected for Short Oral Presentation)

Improving oligodendrocyte maturation in 3D BrainSpheres: Dual optimization via PDGFα supplementation and microglia integration

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Current OECD test guidelines for developmental neurotoxicology, which rely on animal models, face significant limitations in accurately predicting human outcomes. In this context, the human induced pluripotent stem cell (hiPSC)-derived 3D BrainSpheres (BS), containing neurons, oligodendrocytes, and astrocytes, represent a promising human-relevant *in vitro* model. However, oligodendrocyte maturation within BS remains suboptimal, limiting the model's ability to recapitulate key neurodevelopmental processes such as myelination, which is a critical hallmark for brain development.

Therefore, we aimed to enhance oligodendrocyte maturation within BS by generating them from SON hiPSC line and by using two strategies: 1) supplementation with platelet-derived growth factor alpha (PDGFα), known to promote oligodendrocytes progenitors' proliferation and survival, 2) incorporation of microglial cells, which secrete trophic factors that support oligodendrocytes survival, differentiation, and myelination. In detail, PDGFα was added to the standard culture medium at 100 ng/mL from week 4 and week 8 of BS differentiation since PDGF receptors are upregulated from week 4. In parallel, IMR90 DU377 hiPSC-derived microglia (iMG) and human immortalized microglia (HMC3 line) were integrated into BS, either at day 0 or at week 6 of differentiation, using different cell ratios. Finally, microglial integration was assessed using TREM2 immunostaining.

Preliminary findings show that PDGFα supplementation may promote the formation of longer MBP processes, while the presence of microglia, either HMC3 or iMG, may help refine MBP morphology, resulting in more defined and extended processes. While these results are promising, further studies are needed to elucidate the specific effects of microglia presence and PDGFα treatment on oligodendrocytes maturation in the BS.

Keywords: hiPSCs, BrainSpheres, microglia, oligodendrocytes maturation, immunohistochemistry

Poster 13

Establishing an Adverse Outcome Pathway for Developmental Neurotoxicity: Inhibition of Voltage-Gated Sodium Channels leading to Impaired Myelination and Cognitive Decline

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The rising prevalence of neurodevelopmental disorders and potential chemical contributions demand more effective developmental neurotoxicity (DNT) assessments. New Approach Methodologies (NAMs), combined with the Adverse Outcome Pathway (AOP) concept promoted by the Organization for Economic Co-operation and Development (OECD), integrate non-animal data and link molecular events to adverse outcomes, providing a mechanistic and human-relevant alternative to traditional animal studies.

This project aims to establish AOP 489, describing how binding of a compound to voltage-gated sodium channels (VGSCs) during critical windows of oligodendrocyte (OL) development disrupts OL differentiation and myelination, leading to downstream cognitive deficits. This pathway extends the putative EFSA stressor-dependent AOP network for the pesticide deltamethrin, emphasizing OL biology and vulnerable developmental windows.

To support this AOP with scientific evidence, semi-systematic literature reviews and weight-of-evidence analyses were conducted to assess empirical support and biological plausibility of the Key Events (KEs) and KE Relationships (KERs). Moreover, public single-cell RNA-seq datasets were analysed to identify VGSC expression in human glial cells for confirming the human relevance of the AOP by identifying OL progenitors as a vulnerable cell type in humans.

The collected evidence consistently linked VGSC disruption to impaired OL differentiation and hypomyelination supporting the empirical and biological plausibility and establishing causal relationships between the KEs. Single-cell RNA-seq data further confirmed the human relevance and defined the susceptible phase during development for this AOP.

Overall, this study demonstrates the value of integrating *in vitro* phenotyping with omics data, structured evidence evaluation, and mechanistic reasoning to enhance chemical hazard identification while reducing reliance on animal testing. These insights provide a foundation for future development interconnected AOP networks, advancing predictive, efficient, and ethical chemical safety assessment.

Keywords: Developmental Neurotoxicity (DNT), Voltage-gated sodium channel (VGSC) Oligodendrocytes (OLs), Myelination, Adverse outcome pathway (AOP)

Poster 14

Involvement of microglia in developmental neurotoxicity induced by chemicals

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A battery of *in vitro* (IVB) assays, based on human cells and targeting neurodevelopmental key processes, has been assembled with the goal to improve developmental neurotoxicity (DNT) testing. However, microglia are not present in this IVB despite their crucial roles in brain development. Microglia are the brain tissue-resident macrophages. They contribute to key developmental processes such as synaptic pruning, myelination and vasculature formation. Thus, chemicals directly altering homeostatic microglia functions are likely to influence the development of the brain. The goal of this study is to evaluate the direct impact of chemicals on microglia core functions.

In this study, we first characterized two microglia cell models: the immortalized cell line (Human microglia clone 3, HMC3) and human induced pluripotent stem cells (hiPSCs)-derived microglia cell (iMG) differentiated following Chen et al., 2021 protocol. We show by immunostaining that both models are positive for microglia markers (IBA1, TMEM119, TREM2, P2RY12 and PU.1). Both models also respond to 100 ng/ml of lipopolysaccharide (LPS) by increasing mRNA level for interleukin 1 beta (IL1β), tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6). We also exposed the cells to 3 different chemicals: paraquat, amiodarone and propylene glycol phenyl ether. Cell viability showed that the immortalized line is less sensitive than iMG to these compounds. No modifications of pro-inflammatory cytokines (IL1β, TNFα and IL6) was observed at mRNA level at relevant concentrations to human exposure. These results highlight similarities and differences between immortalized and hiPSC-derived microglia. Further research is needed to identify the model that more closely mimics human microglia physiology, to allow for efficient and reliable developmental neurotoxicity testing.

Keywords: microglia, developmental neurotoxicity

Poster 15

Proteomic profiling of DNA adduct binding proteins to elucidate cellular responses to mutagenic DNA lesions

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The risk of developing cancer in response to genotoxic carcinogen exposure may be influenced by DNA binding proteins, which play a crucial role in the recognition and initiation of DNA repair as an adaptive cellular response. However, the proteins involved in damage recognition, except for those involved in some of the most common DNA adducts, are often unknown. For instance, the risk of colorectal carcinogenesis due to high consumption of red and processed meat is partially attributed to error-prone DNA replication at sites containing O^6-carboxymethylguanine (O^6-CMdG), a mutagenic DNA adduct regarded as a potential molecular initiating event of colorectal cancer. Repair of O^6-CMdG in cells appears to rely on both the proficiency in the nucleotide excision repair (NER) pathway and direct removal mediated by MGMT, implying a potential interaction of repair factors. In order to gain further evidence for this as a basis of adduct repair, and to understand how these pathways may interact, the objective of this project is to identify proteins that bind to O^6-CMdG. These may include MGMT, NER factors, or novel as yet unidentified adduct binding proteins (ABP). We investigated interactions of DNA ABPs with O^6-CMdG via a structural proteomics approach called limited proteolysis-mass spectrometry (LiP-MS), which identifies proteins that undergo structural confirmation change upon non-covalent binding. Initially, proteome extracts from multiple cell lines were tested to ascertain the detectability of the anticipated DNA ABPs. Following this, genomic DNA was extracted from human cell lines, fragmented, and chemically exposed in order to introduce specific DNA adducts in a controlled fashion. The resulting modified DNA substrates were subsequently incubated with a protein extract from a human cell line and LiP-MS was applied to identify the putative DNA ABPs. A more profound comprehension of the molecular mechanism underlying O^6-CMdG accumulation and the implementation of LiP-MS to map DNA adduct-protein interactions promise to refine our grasp of exposure-induced carcinogenesis and help to elucidate the role of repair proteins involved the processing of uncommon DNA adducts.

Keywords: LiP-MS, DNA adduct, DNA repair, MGMT, NER

Poster 16

Bisphenol TMC exhibits greater estrogenic activity than Bisphenol A exemplified by higher estrogen receptor α-mediated gene expression and breast cancer cell proliferation

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Bisphenol A (BPA), a widely used monomer in the production of polycarbonate plastics, has raised increasing concern due to its endocrine-disrupting properties. Consequently, it is being progressively replaced by structural and functional analogues. While many of these analogues share close molecular similarities with BPA, their potential endocrine-disrupting effects remain poorly characterized. Among the least-studied BPA analogues are cyclo-di-bisphenol-A diglycidyl ether (cyclo-di-BADGE), tetrabromobisphenol S (TBBPS), bisphenol SIP (BPSIP), and bisphenol TMC (BPTMC). First, we compared their estrogenic activity with BPA. Among the analogues, BPTMC was a significantly more potent ERα agonist than BPA in transactivation assays using HEK-293 cells transiently expressing ERα (EC50 of 87 ± 20 nM vs. 400 ± 100 nM for BPA). *In silico* analyses suggest that the increased potency of BPTMC is due to its greater hydrophobicity and the presence of a bulkier bridging group connecting the two phenolic rings. Unlike BPA, BPTMC also moderately inhibited 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) activity (IC50 of 4.8 ± 0.6 μM), while no significant effect was observed on 17β-HSD1 for any compound. Exposure of ERα-positive MCF-7 breast cancer cells to 1 μM BPTMC for 24 h robustly induced the expression of the ERα target genes GREB1, TFF1, and PGR. The effect was comparable to the induction by 10 nM 17β-estradiol (E2) and was completely abolished by co-treatment with 100 nM of the ERα antagonist fulvestrant. Moreover, BPTMC stimulated the proliferation of estrogen-dependent MCF-7 cells at nanomolar concentrations over 72 h, an effect similarly reversed by 100 nM fulvestrant. Collectively, these findings identified BPTMC as a potent ERα agonist. BPTMC is capable of eliciting transcriptional and mitogenic responses at low concentrations, raising concerns about its safety regarding potential endocrine-disruption and breast cancer-promoting effects.

Keywords: Bisphenol A; Bisphenol TMC; Endocrine disruptor; Estrogen receptor α; Regrettable substitution.

Poster 17

From Checklist to Strategy: A Paradigm Shift in the Biological Evaluation Strategy for a Smarter Biological Risk Assessment

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The biological evaluation of medical devices is undergoing a paradigm shift with the release of the new ISO 10993-1:2025. Moving beyond a prescriptive checklist approach, the current standard adopts a risk-based framework that better aligns with ISO 14971 and current toxicological risk assessment compliant with ISO 10993-17:2023. This shift brings toxicologists into a more strategic position within the medical device lifecycle, expanding their role from testing coordinators to architects of comprehensive biological risk assessments.

The 2025 revision emphasizes the integration of toxicological expertise early in device development and supports a flexible evaluation model. By encouraging the use of existing data, New Approach Methodologies (NAMs), and material characterization, the standard allows industry stakeholders to reduce reliance on traditional animal testing while strengthening the scientific foundation of biological safety. The opportunity to apply methods such as *in silico* modeling, read-across, TTC-based screening, and robust literature evaluations enables a more predictive and tailored assessment of device-specific risks.

This study involved a comparative review of ISO 10993-1:2018 and the newly drafted revision, supported by practical implications of these changes on the design of biological evaluation plans (BEPs). Through a toxicologist's lens, this poster explores the implications of ISO 10993-1:2025 for risk assessors, manufacturers, and regulators. Case-based scenarios illustrate how the revised standard transforms the biological evaluation from a series of tests into a proactive, science-driven strategy. Embracing this opportunity can help stakeholders strengthen their toxicological risk portfolios and enhance healthcare solutions worldwide.

This approach not only facilitates compliance with evolving global regulatory expectations but also opens the door to more efficient development pipelines, cost savings, and innovation in materials and design. Moreover, it empowers manufacturers to build safer, more sustainable medical devices—ultimately contributing to improved patient safety and public health outcomes.

Keywords: ISO 10993-1:2025, toxicological risk assessment, NAMs, medical devices, biocompatibility strategy

Poster 18

Quantitative genomic mapping of oxidative DNA damage with an internal reference standard

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Perturbation of redox equilibrium by metabolic activity or chemical exposure gives rise to oxidative stress, leading to DNA oxidation and genomic instability as key outcomes of toxicant action. 8-oxo-7,8-dihydroguanine (8-oxoG) is the most abundant DNA oxidation product and is elevated in many cancers and neurodegenerative diseases. It can mispair during replication and promote G>T transversion mutations, representing a key molecular event in toxicant-driven adverse outcomes. Yet, dose-response aspects of how different exposure conditions may influence distinct genome-wide 8-oxoG patterns are not known.

Here, we refined our single-nucleotide genomic 8-oxoG mapping method (click-code-seq), to enable quantitative comparison of 8-oxoG patterns amongst differentially exposed cells. Firstly, we demonstrated the capacity of click-code-seq to detect 8-oxoG at single-nucleotide resolution using a site-specifically modified plasmid. The analysis revealed a distinct signal at the expected position, confirming accurate detection of the lesion. We then established an internal normalization strategy for quantitative 8-oxoG mapping by adding a fixed mass fraction of exogenous plasmid DNA to each isolated DNA sample prior to library preparation. This strategy was applied to DNA from cells exposed to 4-nitroquinoline-1-oxide, revealing increases in guanine oxidation by correcting variability in library preparation efficiency and sequencing depth, which was not previously possible.

This new quantitative click-code-seq procedure provides a robust and quantitative platform for comparative mapping of induced oxidative DNA damage under conditions of increasing exposure concentrations. Future applications that quantitatively link chemical exposures with 8-oxoG landscapes are expected to contribute to predictive, mechanism-based approaches for evaluating genotoxicity.

Keywords: 8-oxoG; genotoxicity; DNA damage mapping; spike-in normalization

Poster 19

Predicting Acute Respiratory Toxicity of Poorly Soluble Dusts Using *In Silico* Models

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Acute inhalation toxicity testing (OECD TG 403) is widely used to assess short-term respiratory hazards of airborne substances but relies heavily on animal studies, raising ethical concerns and often overestimating toxicity for poorly soluble dusts. This thesis evaluates the Multiple-Path Particle Dosimetry (MPPD) model as an *in silico* alternative for predicting acute respiratory toxicity.

A dataset of 35 poorly soluble pigment studies with historical *in vivo* data was compiled, and 21 were selected based on Klimisch reliability scores. MPPD simulations used parameters from these studies, including particle size, density, MMAD, GSD, and exposure conditions. Regional deposition fractions (head, tracheobronchial, pulmonary) were computed using MPPD, and airway obstruction ratios were calculated by comparing deposited mass diameters to anatomical airway diameters. Deposition patterns across airway generations were also modeled, with obstruction ratios calculated for each generation to assess breathing impairment throughout the lung.

Results showed that more than 50% of the particles deposited in the head region, while deeper lung regions received smaller fractions. Early airway obstruction did not consistently predict mortality. However, deposition in later generations, particularly generation 19, correlated more strongly with lethal outcomes. Deposition was inhomogeneous, with noteworthy accumulation in the upper right lung lobe, explaining survival at high doses despite large total lung burdens.

These findings indicate that *in silico* models like MPPD can refine inhalation toxicity predictions and reduce reliance on animal testing. By demonstrating that *in silico* models can predict critical deposition patterns, it advances non-animal testing methods (NAMs), improves mechanistic insight, and lays groundwork for more human-relevant risk assessments.

Keywords: Acute inhalation toxicity; MPPD model; Non-animal testing methods (NAMs); Particle deposition; Respiratory risk assessment

Poster 20

***In vitro* evaluation of Iranian traditional medicinal plants for the discovery of novel therapeutics for Alzheimer's disease**

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder whose prevalence has risen significantly in recent years. The precise mechanisms underlying its pathogenesis are still unknown, making it a subject of considerable scientific investigation. While no cure currently exists, recent progress in slowing the course of the disease and addressing symptoms has involved the use of disease-modifying therapies targeting amyloid-beta (Abeta) plaques, alongside traditional pharmacological treatments for cognitive and behavioral deficits, which require rigorous safety and neurotoxicity assessment. The use of iranian traditional medicine (ITM) can complement these current conventional strategies to enhance cognitive functions but also requires human-relevant toxicological evaluation to establish tolerated concentration ranges and identify potential neurotoxic effects.

This study aimed to evaluate the effects of selected medicinal plants used in ITM, based on scientific data retrieved from the Noor database using the Persian terms "Nesyan" and "Litharghas" as translations for "memory loss" and validated by ITM experts. Cytotoxicity of the plant extracts was determined in human neuronal progenitor cells (ReNcell VM) the determined IC_{50} and IC_{20} concentrations were then applied to the human ReNcell VM line at distinct neuronal developmental stages: neural progenitors, differentiating cells, and 1-week old neurons to capture a comprehensive view of extract actions. Furthermore, six-week differentiated ReN cells carrying familial AD (ReN-FAD) were evaluated at these sub-cytotoxic concentrations for survival, neuronal differentiation, and Aβ production.

The herbal extracts *Zingiber officinale* Rosc. and *Cyperus longus* L. showed positive effects on the cells. In ReN-FAD differentiated neurons, *Zingiber officinale* Rosc. improved cell viability and neuronal differentiation and possibly reduced Abeta levels compared to healthy parental neurons.

Despite the need for further in-depth investigation to clarify their pharmacological properties and clinical relevance, these two herbal extracts were non-toxic at the tested concentrations and exhibited noteworthy promise for future AD studies and may contribute to the development of integrative medical approaches aimed at mitigating cognitive decline.

Keywords:

Poster 21 (selected for Short Oral Presentation)

AI-Powered Virtual Control Groups: Augmenting Toxicology Analysis

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The pharmaceutical industry is increasingly focused on reducing animal usage due to ethical and regulatory concerns, particularly for non-rodent studies. Virtual Control Groups (VCGs) offer a scalable and cost-effective alternative by leveraging historical control data to replace concurrent controls, with the potential to reduce animal use in toxicology studies by up to 25%.

Our study uses AI and statistical methods to explore reliable approaches to develop and implement VCGs in clinical pathology research, focusing on 3 key areas:

1. Historical Control Database: We generated a comprehensive database from standardised toxicology datasets (2019 onward), including clinical pathology data (haematology, clinical chemistry, coagulation) from 1080 rat and 380 dog studies. Statistical analysis identified key covariates, such as age, sex, and body weight, that influence lab test results. While histopathology data is not yet incorporated, it remains a priority for future framework refinement.

2. VCG Matching: Our goal is to accurately match virtual controls to treated animals in a study, minimising bias and improving the consistency of experimental outcomes. To do this, we apply data-driven methods like k-nearest neighbours and propensity score matching, using covariates like age, sex, body weight, and pre-dose lab data. These strategies are designed for scalability, enabling integration into routine workflows across the industry.

3. Control-Likeness Scoring: To assess the similarity between treated and control animals, we calculate "control-likeness scores" based on lab test data and key covariates. These scores capture deviations from control baseline distributions, aiding pathologists in early detection of potential treatment effects. Using Kernel Density Estimation, we model lab test data distributions while incorporating metadata, offering an early alert system to guide further analysis.

These approaches demonstrate the potential to reduce reliance on concurrent control groups while retaining experimental reliability and reproducibility. Future work will focus on model refinement, external validation, and deployment to strengthen treatment-effect analyses. Our scalable, data-driven approach offers a significant step toward reducing animal usage and augmenting toxicology research.

Keywords: Virtual Control Groups, AI Methodologies, Animal Testing Reduction

Poster 22

Profiling Oxidative DNA Damage at Single-Nucleotide Resolution in Alzheimer's Disease Brains

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, neuronal loss, and the accumulation of amyloid plaques and tau tangles. While specific genetic mutations have been identified in AD, their etiology and contribution to neurodegeneration remain poorly understood. Somatic mutations and mutational mosaicism in neurons have been observed, but the mechanisms driving these mutations, particularly the role of oxidative DNA damage, are unclear. Increasing evidence suggests that oxidative stress and DNA damage, including elevated levels of lesions such as 8-oxo-7,8-dihydroguanine (8-oxoG), may contribute to genomic instability in AD. However, the direct relationship between oxidative DNA damage and disease-associated mutations remains to be fully explored.

This study aims to elucidate this connection through high-resolution single-nucleotide mapping of oxidative DNA damage, providing critical insights into the mechanisms of neurodegeneration and revealing potential therapeutic targets for AD. As part of this research, enzymatic treatment is optimized and evaluated to reduce artifactual background damage while preserving original oxidative DNA modifications, thereby improving the specificity and reliability of 8-oxoG mapping.

Keywords: DNA Damage, Oxidative damage, Alzheimer's Disease

Poster 23

Compositional Profiling-based risk assessment on medical devices composed of biodegradable polymers

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Biodegradable polymers (BPs) present unique challenges for chemical characterization and toxicological risk assessment (TRA), particularly for devices with prolonged or long-term tissue contact.

Exhaustive extraction is impractical for many BPs due to their degradability and incompatibility with common solvents. Additionally, quantifying oligomeric degradation products is analytically complex and may not significantly improve risk estimates compared to worst-case assumptions.

A hybrid approach to TRA for BPs is proposed. It is beginning with compositional profiling to estimate the total quantity of each polymer in a device and applying worst-case assumptions for complete hydrolysis into monomeric constituents. These monomers, being smaller and more reactive than oligomers, represent the most toxicologically relevant degradation products. Estimated exposure doses (EEDmax) were calculated for acute, subacute, subchronic, and chronic durations using assumed release per ISO 10993-17:2023. Where initial margins of safety (MOS) were low (specifically for glycolic acid (GA), a common hydrolysis product of both PLGA and GCTMC) refined exposure estimates were derived using in *vitro–in vivo* correlation (IVIVC) data. These degradation-based release kinetics provided more realistic exposure scenarios, resulting EEDmax values that were less than applicable derived TIs. This approach demonstrates that compositional profiling, supplemented with IVIVC when needed to refine exposure estimates, offers a practical and conservative framework for assessing systemic toxicological risk posed by BPs. These methods only account for BP degradation products and must be supplemented with additional methods to address manufacturing residues.

The poster was presented at SOT'25 Orlando, USA.

Keywords: Toxicological risk assessment (TRA), ISO-10993-17, compositional profiling, biodegradable polymers (BP), chemical characterization

Poster 24

Toward Regulatory Confidence in NAMs for Endocrine Disruption: Scientific Promise and Practical Gaps Across EU Sectors

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The EU's shift toward non-animal, mechanistic chemical safety assessment places New Approach Methodologies (NAMs) at the forefront of regulatory toxicology. Endocrine disruption (ED), especially via thyroid-related mechanisms, remains a scientifically and regulatory complex issue across multiple sectors, including agrochemicals, biocides, industrial chemicals (REACH) and pharmaceuticals.

Limitations of traditional *in vivo* testing and species-specific differences make thyroid related assessments particularly complex. NAMs such as *in vitro* assays, *in silico* models and omics approaches can provide mechanistic insights and enhance the evaluation of human relevance. However, their acceptance in regulatory decision-making is still limited. In line with current regulatory trends, EFSA and ECHA encourage the use of weight-of-evidence and next-generation risk assessment (NGRA), while OECD test guidelines were recently updated to include relevant ED endpoints. Still, validated NAMs for ED assessments, particularly for thyroid disruption, are lacking and regulatory acceptance remains in its early stages.

Efforts are underway to support the integration and validation of NAMs through EU initiatives such as EU-NETVAL, PARC, the EURION and ASPIS cluster. A coordinated strategy based on Adverse Outcome Pathways (AOPs), species comparison and early dialogue is essential to foster regulatory confidence across sectors.

Keywords: New Approach Methodologies (NAMs), endocrine disruption, regulatory toxicology, AOPs, 3Rs.

Poster 25

Building a high-throughput method for computationally predicted AOP

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The Adverse Outcome Pathway (AOP) framework was developed to organize knowledge between stressor non-specific exposures and an adverse outcome as a sequence of events. AOPs explain how chemical exposures can lead to an Adverse Outcome (AO). This concept links a Molecular Initiating Event (MIE) as the first trigger of an outcome to one or several Key Events (KE) and, ultimately, one AO. However, standard AOP development methods depend on manual curation and experimental steps, which are labor intensive and time consuming. As such, there are very few AOPs available. A high-throughput method for AOP generation using publicly available data from various databases would offer new opportunities for rapid development of AOPs and increase our understanding of potential adverse effects from chemical exposure.

We present a computational workflow for AOP development focused on insecticides and Parkinson disease in humans as a proof of concept. Our method bridges diverse databases (e.g., The Human Proteome Atlas, Bgee) using a pipeline to integrate, filter and structure these data according to the AOP framework. Gene Ontology and Reactome terms are used as the core block of the KEs, Comparative Toxicogenomics Database pesticides-genes interactions represent the MIEs and, Parkinson Disease serves as the AO. This pipeline results in thousands of candidate AOPs that can be ranked to prioritize which pesticides and mechanisms should be further assessed. Some relevant KEs our pipeline identified across pesticides in the most shared AOPs, highlight an impact on the neuronal apoptotic process, and associated genes are implicated as the MIE of those AOPs. Additionally, the database DISGENET provides information about known genetic variants implicated in Parkinson disease. Integrating these variants into our workflow provides insight into gene-environment interactions from pesticide exposure, which would help provide a population-relevant perspective to AOPs for chemical risk assessment.

Keywords: Adverse Outcome Pathway, Computational approach, Chemical risk assessment, Data integration

Poster 26

Integrating the 3R Principle and STOP Strategy in Occupational Toxicology for Feed and Fragrance Intermediates

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Occupational exposure to the structurally related feed and fragrance intermediates Isopropenyl Methyl Ether (IPM) and Butenyl Methyl Ether (BME) are potentially critical, particularly via inhalation in industrial environments. To establish robust internal occupational exposure limit (IOEL), addressing both systemic toxicity and local respiratory effects, a multidisciplinary approach guided by the 3R principle was employed.

Firstly, systemic effects were assessed through an *in vivo* oral 28-day study in rat with IPM, based on which a systemic IOEL was derived. For evaluation of local toxicity, *in vitro* studies were prioritized by using the human 3D airway epithelial tissue model MucilAir™-Pool Bronchial, which simulates repeated exposure via inhalation. In this assay, IPM and BME at concentrations equivalent to airborne exposures of 40, 80, and 120 ppm were applied once per day over four consecutive days. The selected concentrations correspond to 1-, 2- and 3-times the derived systemic IOEL for IPM. Endpoints evaluated included tissue integrity (TEER), cytotoxicity (LDH release), pro-inflammatory response (IL-8 secretion), cilia beating frequency (CBF), and mucociliary clearance (MCC). Results demonstrated no significant changes in tissue viability, barrier function, or mucociliary clearance at any tested concentration for either substance, and no increases in LDH or IL-8 levels, indicating the absence of cytotoxic or inflammatory effects.

These findings confirm that exposure via inhalation to IPM at or above the systemic IOEL of 40 ppm does not compromise bronchial epithelial health. Applying a read-across principle for the structurally similar BME, an IOEL of 34 ppm was derived, which covers also the absence of local effects from exposure via inhalation.

This project demonstrates the value of integrating *in vivo* and *in vitro* toxicology with occupational hygiene to derive health-based OELs and ultimately comply with the well-established hierarchy of controls or STOP principle*, prioritizing technical controls in occupational settings. It highlights the applicability of the new approach methodology (NAM) testing strategies and the importance of collaboration between toxicology and industrial hygiene in protecting worker health and ensuring regulatory compliance.

*STOP principle: S – substitution, T – technical controls, O – organizational controls, P – personal protective equipment

Keywords: Occupational Exposure Limits (OELs), Industrial Hygiene, Isopropenyl Methyl Ether (IPM), Butenyl Methyl Ether (BME), New Approach

Methodologies (NAMs) Poster 27

Effects of chemically induced oxidative DNA damage on transcriptional regulation

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DNA oxidation, including the formation of 8-oxoguanine (8-oxoG), is a hallmark of oxidative stress and represents a key form of DNA damage arising from chemical or environmental exposures that threaten genome stability and cellular functions. Such lesions can influence transcription regulation when present at specific promoter regions, such as G-quadruplex structures. However, the consequences of genome-wide accumulation of oxidative DNA damage on gene expression, and how DNA repair pathways mitigate these effects, remain poorly understood. Thus, we combined click-chemistry-based DNA damage sequencing with gene expression and chromatin accessibility profiling to map guanine oxidation sites at single-nucleotide resolution. We further investigated how the loss of CSB, a key sensor protein of transcription-coupled nucleotide excision repair (TC-NER), modulates the relationship between guanine oxidation patterns and gene expression.

By mapping 8-oxoG in a human cell line exposed to potassium bromate, we observed a transcription-dependent strand bias, whereby there were higher oxidation levels on the non-transcribed strand compared to the transcribed strand within gene bodies. While this bias was independent of repair loss of repair capacity disrupted downstream gene expression.

We further found that guanine oxidation increases in specific genomic regions but is largely repaired within 24 hours.

These data suggest a strand-selective mechanism whereby oxidized guanines accumulate in highly expressed genes and may function to prevent transcription-associated genotoxic stress.

Together, our findings highlight the dynamics of DNA damage in shaping transcriptional and stress-related cellular responses to oxidative stress, opening avenues to explore how chemical and environmental exposures influence genome function and cellular adaptation.

Keywords: Guanine oxidation, DNA damage sequencing, Oxidative stress

Poster 28

Human gut microbes transform the chloroacetamide herbicide metazachlor to metabolites with altered toxicological characteristics

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Metazachlor, a globally used chloroacetamide herbicide, is characterized by its environmental persistence and hydrolytic stability. It undergoes microbial biotransformation in the environment, but its metabolism by human gut microbiota remains unexplored. Uncovering how gut microbes modify metazachlor is essential for understanding potential alterations in its toxicity, bioavailability, and systemic exposure in humans.

Here, we characterized how metazachlor is metabolized by human gut microbiota and assessed potential toxicological risks presented by its metabolites. We exposed human gut microbial communities to metazachlor at concentrations based on the EFSA-derived acceptable daily intake (ADI) level. Using untargeted LC-MS/MS analysis, we identified 6 known and 18 previously unreported metabolites of metazachlor and annotated these on the basis of putative biotransformation pathways. The most abundant primary metabolite in gut microbiota incubations was a cysteine–metazachlor adduct formed abiotically, representing a distinct transformation route from the glutathione-based metabolism reported for soil and aquatic microbiota. Furthermore, we synthesized several key proposed metabolites and used them as reference standards to elucidate the multi-step process of metazachlor gut microbial degradation and metabolite formation.

Next, the cytotoxicity of key metabolites was characterized using human gastrointestinal cell lines, and two metabolites were more cytotoxic than metazachlor. We confirmed that one of these had high cell permeability, similar to metazachlor. To assess human health risk, we developed a physiologically based pharmacokinetic (PBPK) model and predicted blood concentrations of metazachlor and its key metabolites. The model indicated fast excretion of metazachlor and showed that predicted metabolite levels are below cytotoxicity thresholds at the ADI level of metazachlor consumption.

Our findings reveal that metazachlor is extensively metabolized by the human gut microbiota to metabolites potentially posing toxicological risks, suggesting their consideration in evaluating human health risks due to metazachlor exposure.

Keywords: metazachlor, gut microbiota, metabolism, LC-MS^2

Poster 29

Inhibition of Non-Drug-Metabolizing CYP450s: Interference of steroidogenesis by azole antifungals

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Novel small-molecule drugs must be assessed for their drug–drug interaction (DDI) risk. Such screenings always include key cytochrome P450 (CYP) isoforms such as CYP3A4, CYP2C9, and CYP2D6, since their inhibition can elevate co-medication exposure. In contrast, CYP enzymes that synthesize or transform endogenous molecules such as steroid hormones, vitamins, or retinoids are not systematically profiled, although their inhibition can cause predictable and clinically relevant toxicity rather than classical PK interactions.

We evaluated clinical triazole antifungals (itraconazole, fluconazole, posaconazole, voriconazole, isavuconazole), which are known CYP inhibitors, and newer tetrazoles (oteseconazole, VT-1129, VT-1598), which were designed for higher selectivity toward fungal CYP51. Compounds were tested in the human adrenal H295R cell line to assess effects on adrenal steroidogenesis and in plasmid-transfected cell lines overexpressing individual steroidogenic enzymes to quantify inhibitory potential. Steroid profiles in H295R supernatants were measured by targeted and untargeted LC-MS/MS panels of glucocorticoids, mineralocorticoids, progestins, and androgens in the presence of multiple concentrations of test compounds.

Posaconazole potently inhibited CYP11B1, to a lesser extent 11β-HSD2, and showed weaker CYP17A1 inhibition. Itraconazole potently inhibited 11β-HSD2, and to a lesser extent CYP11B1. These activities align with the described pseudohyperaldosteronism phenotype characterized by hypertension, hypokalemia, low renin, and suppressed aldosterone. These findings indicate that azoles act beyond fungal CYP51 and hepatic CYP3A4 and can directly alter human adrenal steroidogenesis. Our results reveal a gap in current safety assessment practices as inhibition of non-drug metabolizing CYP450 enzymes is experimentally accessible and clinically relevant.

Keywords:Adrenal steroidogenesis, Azole antifungals, CYP450 inhibition, Pseudohyperaldosteronism

Poster 30

Application of a quantitative approach to identify Poorly Soluble Particles

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Recent regulatory proposals and decisions have reignited discussions surrounding inhalation of Poorly Soluble, Low Toxicity Particles, emphasizing the need for precise definitions and questioning the applicability of the Classification, Labelling, and Packaging regulation. In response, an ECETOC Task Force was formed to specify criteria for "poorly soluble" particles (PSP) and to identify possible PSP in a defined data set. Furthermore, a quantitative method for defining PSP was established using a model particle, focusing on their potential to cause volumetric lung overload and affect pulmonary macrophage clearance mechanisms. Finally, existing dust limits were used to challenge the established PSP threshold.

The Task Force collected data, including information from member companies and publicly available literature, to identify patterns in the effects of sub-acute and sub-chronic inhalation studies in rats and the No Observed Adverse Effect Concentrations (NOAEC) derived. The final data set comprised nineteen OECD guideline 412 or 413 studies on organic and inorganic compounds. Lung burdens were calculated for each substance by the Multiple-Path Particle Dosimetry Model examining possible correlations between lung burden and adverse effects observed. Based on Morrow`s overload hypothesis a threshold of the dissolution rate for PSP was calculated and, in a proof-of-concept approach, challenged regarding their suitability for PSP using the derived threshold and MPPD calculated human lung burden.

None of the investigated substances demonstrated systemic toxicity; however, pulmonary foreign body inflammatory reactions following sub-chronic or sub-acute exposure were observed, regardless of their chemical composition.

The analysis of the model particle indicates that a dissolution rate exceeding 0.057 per day would not lead to a lung burden greater than 1 µL/g of lung tissue over a sub-chronic study in rats at 20 mg/m³. Most substances of the data set exhibited dissolution rates below this critical threshold. Also, the lung burden at NOAEC remained below the limit for lung overload. ZnO demonstrated a higher dissolution rate and a lower NOAEC compared to the other substances assessed. BaSO4 however was identified as borderline material.

The scrutiny of the MAK dust limit and the recommended ECHA value for insoluble dusts against the derived PSP

threshold demonstrated that both serve as effective protective limits for exposure to PSPs. Consequently, the established threshold emerges as a valuable tool for a quantitative definition of the term PSP. The current CLP guidance values for inhalation toxicity testing and classification apply, however, not adequately so to PSP. Instead, the existing human dust limits provide a more appropriate alternative for safeguarding human health.

Keywords:

Poster 31

New Approach Methodologies (NAMs) Using Fish Cell Lines to Assess Chemicals and Tire-Derived Microplastics

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Microplastic contamination is an increasing environmental concern, with potential adverse effects on both humans and wildlife health. Tire and road wear particles (TRWPs), generated by tire abrasion on road surfaces, represent a major source of microplastics that enter the environment through runoff and release chemicals posing significant ecological risks. Among these, 6PPD-quinone (6PPD-Q), formed by the environmental oxidation of the tire wear antioxidant 6PPD, has been identified as a key contributor to urban runoff mortality syndrome (URMS), responsible for the mass death of coho salmon on North America's west coast.

To investigate the toxicological effects of tire-derived pollutants, we applied an established *in vitro* cell-based toolbox from rainbow trout (Oncorhynchus mykiss) consisting of RTgill-W1, RTgutGC, and RTbrain cell lines, derived from gill, intestine, and brain tissues, respectively. This method, initially developed for RTgill-W1 and validated in OECD Test Guideline Nº 249, was used to assess the acute toxicity of particles and chemicals by evaluating cell metabolic activity, lysosomal function, and membrane integrity. Interestingly, we found that 6PPD-Q was particularly toxic to RTbrain cells but not to the other lines.

To explore molecular responses, transcriptomic and proteomic analyses were performed, showing dysregulation of pathways related to oxidative stress, quinone detoxification, biotransformation enzymes, tight junctions, and inflammation—findings consistent with previous studies on 6PPD-Q toxicity. Although the exact mechanism of action still remains unclear, these fish cell lines provide a practical and mechanistically informative system for screening tire-related pollutants. Overall, this *in*

vitro approach advances the understanding of tire chemicals toxicity to aquatic organisms while supporting the use of New Approach Methodologies (NAMs) that reduce the need for animal testing.

Keywords:

Poster 32

The impact of gut microbiota on the metabolism and toxicity of fluorinated pharmaceuticals

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Fluorinated pharmaceuticals are increasingly used in pharmacology and currently account for approximately 20% of all approved drugs. Their favorable pharmacokinetic properties, including enhanced bioavailability and metabolic stability, arise partly from the strong carbon–fluorine bond, which confers resistance to metabolic degradation. Consequently, microbial transformation of fluorinated compounds has been widely assumed to be limited or negligible. However, preliminary observations indicate that certain gut microorganisms can still modify these compounds, albeit slowly, suggesting that microbial metabolism of fluorinated pharmaceuticals may be more prevalent than previously thought and warrants systematic investigation.

Therefore, we used microbiota from healthy human donors in a high-throughput fecal fermentation system coupled with LC–MS/MS analysis to screen 32 fluorinated compounds for gut microbial biotransformation. We found that flutamide and fluazinam were rapidly biotransformed in all donors, while pretomanid degradation increased with higher fecal slurry concentrations. Untargeted LC–MS/MS analysis revealed that flutamide forms a metabolite that was previously reported to be formed by the human liver, but not as a transformation product of gut microbial metabolism. To obtain preliminary toxicological information, we used the quantitative structure-activity relationship (QSAR) model from the Danish QSAR Database. The predicted biological activity of the flutamide metabolite suggested a potential interaction through antagonism of androgen receptors. In addition to the flutamide-derived metabolite, seven fluazinam and four pretomanid metabolites were identified.

These findings demonstrate that fluorinated pharmaceuticals undergo microbial biotransformation by the human microbiome. They also highlight the potential for gut microbiota to influence inter-individual variability in xenobiotic metabolism and safety.

Keywords: biotransformation, pharmacokinetics, microbial metabolism, fluorinated drugs, toxicity