Annual Meeting 2026 – Poster Session

Poster 01

Distinguishing Adult and Developmental Neurotoxicity Using New Approach Methodologies: The PARC project NAM4NT

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The transition towards animal-free chemical safety assessment raises a fundamental question for neurotoxicity: can New Approach Methodologies (NAMs) not only identify neurotoxic effects, but also determine whether they represent adult neurotoxicity (ANT) or developmental neurotoxicity (DNT)? This distinction is important across regulatory frameworks, including REACH, CLP and the Biocidal Products Regulation, yet it is complicated by substantial overlap in the underlying biology and the evidence generated by current test methods. Within the European Partnership for the Assessment of Risks from Chemicals (PARC), the project NAM4NT aims to evaluate whether neurotoxicity NAMs are able to distinguish between ANT and DNT. The project follows three connected pillars: (i) mapping relevant biology, mechanistic domains and adverse outcome pathways (AOPs); (ii) integrating and reanalysing available neurotoxicity data; and (iii) developing targeted case studies using complementary human cell-based and zebrafish NAMs. I will present the project strategy and outline how mechanistic knowledge, comparative analyses of reference chemicals and NGRA-style case studies could inform the ANT–DNT distinction. Overall, this work addresses what NAM-based testing strategies need to deliver to support ANT and DNT assessment, and whether current regulatory frameworks can accommodate this evidence or must evolve alongside the methods.

Poster 02

Human Health Risk Assessment of Metformin in Drinking Water Based on Frequent Monitoring Data: A Case Study in Lausanne

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Metformin is increasingly recognized as emerging contaminant in drinking water systems worldwide because of its very high global consumption, poor metabolism in humans, and frequent detection and persistence in aquatic environments. Recent monitoring data from the Lausanne Water Service (Service de l'Eau de Lausanne) confirmed its continuous presence in drinking water. Given the lifelong daily consumption of drinking water and the potential for chronic low-dose exposure, the objectives of this case study were to a) characterize the occurrence and concentration range of metformin in treated drinking water in Lausanne, b) assess lifelong exposure of consumers via drinking water, and c) evaluate the potential human health risks associated with this exposure.

A screening-level human health risk assessment was conducted using the standard framework and monitoring data from the Lutry and St. Sulpice drinking-water treatment plants for 2020–2025. Scientific and regulatory literature supported hazard identification and characterisation. Risk was assessed using hazard quotient (HQ) and margin of exposure (MOE) approaches.

Metformin was the most frequently detected pharmaceutical and reached concentrations of 5–203 ng/L (mean: 51.46 ng/L), with incomplete removal during treatment (46–86%). Lifetime exposure was approximately 10.6 mg over 70 years, corresponding to ~2.1% of a minimum therapeutic dose and ~0.5% of the WHO defined daily dose, indicating very low exposure relative to clinical use. Although metformin is generally considered safe at therapeutic doses, evidence of potential endocrine and reproductive effects was considered in the hazard characterisation. HQ values were ≤ 0.005, approximately 200-fold below the threshold of 1, indicating that exposure is unlikely to result in adverse health effects. Margins of exposure ranged from approximately 2×10^5 to 1.4×10^6 greatly exceeding commonly used benchmark values (e.g. 100 or 1000), and indicating a very large margin of safety.

The results indicated that metformin concentrations detected in drinking water supplied by the Lutry and St. Sulpice treatment plants do not pose an appreciable risk to human health. Nevertheless, the frequent detection of metformin reflects its widespread use and environmental persistence. With the increasing prevalence of type 2 diabetes in Switzerland, concentrations may rise, highlighting the importance of continued surveillance and of diabetes prevention as a complementary, source-level measure. Future work should include monitoring of transformation products such as guanylurea, consideration of mixture effects, and further evaluation of treatment technologies capable of improving the removal of highly polar pharmaceuticals from drinking water.

Keywords: metformin; drinking water; human health risk assessment; pharmaceuticals; emerging contaminants

Poster 03 (selected for Short Oral Presentation)

Leveraging low-input RNA-sequencing in human and animal liver microtissues to identify species- and compound specific mechanisms of drug-induced liver injury

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Drug-induced liver injury (DILI) remains a major challenge in drug development, often emerging late in clinical trials. In addition, hepatotoxic responses observed in nonclinical species do not always translate to humans, complicating regulatory decision-making and human risk assessment.

To evaluate the utility of species-specific liver microtissues (LiMTs) as a translational platform for identifying mechanistic differences in drug-induced liver toxicity across compounds and species, low-input bulk RNA sequencing was applied to LiMTs derived from human, cynomolgus monkey, beagle dog, and Sprague Dawley rat. These physiologically relevant 3D models, composed of primary parenchymal and non-parenchymal species-specific liver cells, were exposed to troglitazone, rosiglitazone, or fialuridine – compounds with well-characterized and distinct hepatotoxicity profiles. Following 7 days of exposure, cytotoxicity and transcriptomic responses were assessed.

Troglitazone and rosiglitazone are PPARγ agonists formerly used for type 2 diabetes, however, troglitazone was ultimately withdrawn due to severe hepatotoxicity. In human LiMTs, both drugs induced PPARγ-related pharmacological signatures, whereas only troglitazone triggered pathways associated with bile acid dysregulation, oxidative stress, apoptosis, and electrophilic stress, highlighting distinct DILI-related mechanisms.

Fialuridine, a nucleoside analog associated with human-specific hepatotoxicity, induced pronounced cytotoxicity in human LiMTs but not in animal-derived LiMTs, consistent with in vivo findings. Transcriptomic analysis revealed a human-specific gene expression signature involving DNA damage response, mitochondrial dysfunction, bile acid metabolism, and detoxification pathways, in line with the reported mechanisms of fialuridine-induced liver injury in vivo.

In conclusion, species-specific LiMTs combined with transcriptomic profiling provide a translational platform to identify compound- and species-dependent mechanisms of liver toxicity. This approach enhances the sensitivity of liver microtissues for DILI risk assessment at clinically relevant exposure levels and has the potential to improve human risk prediction and bridge interspecies differences in drug safety evaluation.

Keywords: Liver microtissues, transcriptomics, Drug-induced liver injury (DILI), species-specific, species-dependent

Poster 04

When is good, good enough? Transcriptomic assessment of model performance in *in vitro* neurodevelopmental toxicity testing

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Microglia support neural development and maintain brain homeostasis throughout life, and their dysfunction has been implicated in neurodevelopmental disorders. Current neurotoxicity testing strategies, including the developmental neurotoxicity (DNT) test battery, do not include microglial models. This may lead to an incomplete assessment of neurotoxic outcomes of direct and indirect microglia-mediated effects. Human iPSC-derived microglia (iMG) are increasingly employed as human-relevant models, but are generated using diverse differentiation protocols. It remains unclear how closely these models resemble primary human microglia in terms of molecular identity and function, and how they compare with immortalized cell lines.

This study compiles public and newly generated transcriptomic datasets capturing microglial responses to *in vitro* chemical exposures, including iMG generated using diverse differentiation strategies: embryoid body-, transcription factor overexpression-, and coculture-based protocols, and immortalized microglial/monocytic cell lines (HMC3 and THP-1). Reference signatures of microglial maturation and of homeostatic and disease-associated states are derived from public bulk and single-cell transcriptomes of primary fetal and adult microglia. Microglial identity, state, and responses to chemical exposure are characterized, focusing on immune activation and AOP-relevant processes.

Differentiation protocols generate distinct microglial states at the transcriptomic level, suggesting that the most appropriate iMG model depends on the biological question addressed. Models of varying complexity show increased gene set scores upon LPS stimulation, yet the genes driving this differ between HMC3 and iMG. By integrating additional differentiation protocols and chemical exposures, this framework will establish the level of model complexity required to capture toxicologically relevant microglial responses, thereby guiding model selection for neurotoxicity testing.

Keywords: Microglia, Developmental neurotoxicity, *In vitro* toxicology, Transcriptomics

Poster 05

An animal-free strategy to bridge *in vitro* bioactivity and human exposure for the emerging mycotoxin beauvericin

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Beauvericin (BEA) is an emerging cyclodepsipeptide mycotoxin produced by Fusarium fungi and constitutes a potential public health concern due to its natural occurrence in crops and feeds. BEA has been associated with *in vitro* cytotoxicity and nuclear receptor-mediated effects at micromolar concentrations. Whether such concentrations are reached at target sites under realistic dietary exposure scenarios is considered a crucial data gap for chemical risk assessment.

We addressed this gap through an animal-free approach integrating in silico prediction, *in vitro* testing and physiologically based kinetic (PBK) modelling. BEA was not transformed by gut microbiota but underwent phase I metabolism in hepatic microsomes. The hepatic biotransformation products were evaluated by LC-MS/MS analysis. Apparent intestinal permeability was determined in Caco-2 monolayers. The parameters obtained were integrated into a physiologically-based kinetic (PBK) model, evaluated against available rodent toxicokinetic data and further extrapolated to the human organism. The predicted blood C_{max} value closely matched experimental results, while the predicted area under the curve (AUC) and peak time were within the 1.6-fold. QSAR tools were used to predict receptor agonism or antagonism, and flagged endpoints were assessed in an OECD-validated reporter-gene battery (ERα and AR CALUX® assay) extended to TRβ, PXR, PPARγ and DRhuman CALUX, with and without S9-mediated bioactivation. Estrogenicity was also assessed by alkaline-phosphatase induction in Ishikawa cells, and cell viability was determined in parallel to each assay. Receptor-mediated responses were only observed at concentrations approaching the cytotoxic range, complexifying their attribution to receptor antagonism. The highest concentration maintaining 80% cell viability was therefore used as a conservative point of departure and compared with PBK-predicted concentrations in organs of toxicological interest.

The final aim is to evaluate whether BEA alone can reach systemic levels of human health concern under realistic exposure scenarios.

Keywords: beauvericin, endocrine disruption, PBK modelling, QIVIVE, *in vitro* toxicity testing

Poster 06

TSAssistant: A Human-in-the-Loop Agentic Framework for Automated Target Safety Assessment

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Target Safety Assessment (TSA) requires systematic integration of genetic, transcriptomic, target homology, pharmacological, and clinical data to evaluate potential safety liabilities of therapeutic targets. This process is labor-intensive and expert-dependent, posing challenges in scalability and reproducibility.

We present TSAssitant, a human-in-the-loop multi-agent framework that decomposes TSA report generation into a workflow of specialized subagents: Research Subagents that each ground and cite a single TSA domain, andSynthesis Subagents that integrate findings across domains. Subagents retrieve and synthesize evidence from curated biomedical sources through standardized tool interfaces and produce individually citable, evidence-grounded sections, with behavior shaped by a hierarchical instruction architecture that separates coordination logic from domain expertise and user intent. To complement these soft constraints, programmatic execution hooks and persistent memory stores enforce hard constraints across the workflow, while an interactive refinement loop allows experts to review and revise individual sections with full conversational context preserved across iterations. Rather than a single holistic comparison, we decompose report quality into reproducibility, evidential grounding, task-level accuracy, and controllability under expert oversight, finding high reproducibility and grounding, substantial agreement with the human reference, and net-positive expert-driven refinement.

Poster 07

3D InSight Drug-Induced Gastrointestinal Toxicity (DIGIT) Platform

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Drug-induced gastrointestinal toxicity (DIGIT) is a major cause of dose-limiting adverse events, treatment discontinuation, and late-stage drug attrition, highlighting the need for more predictive and human-relevant preclinical models. Conventional two-dimensional *in vitro* systems lack the structural and cellular complexity of the human intestinal epithelium, limiting their ability to accurately predict gastrointestinal (GI) safety risks. The objective of this study was to demonstrate the utility of the 3D InSight™ *In Vitro* Gastrointestinal Toxicology platform, a patient-derived intestinal organoid-based assay designed to enable earlier and more confident assessment of GI liabilities. Adult multipotent intestinal stem cells isolated from human intestinal biopsies were expanded as region-specific organoids and maintained as long-term biobanked cultures. Using Gri3D® hydrogel microcavity plates, compounds were screened in dose-response formats across up to 35 organoids per well, enabling high-content analysis at single-organoid resolution. GI toxicity was evaluated after 48 hours of compound exposure using a multiplexed approach combining image-based Live/Dead analysis, organoid morphology and size measurements, intracellular ATP quantification, and lactate dehydrogenase (LDH) release. The platform successfully detected toxicity induced by model compounds with distinct mechanisms of gastrointestinal injury and generated reproducible, concentration-dependent responses across multiple endpoints. To date, more than 20 reference compounds with known GI safety profiles have been evaluated, supporting the robustness and predictive potential of the assay. Furthermore, the availability of organoids from multiple species enables cross-species comparisons, enhancing translational relevance and facilitating the assessment of human-specific responses. These results demonstrate that patient-derived intestinal organoids provide a scalable and physiologically relevant platform for GI safety testing, supporting earlier identification of gastrointestinal risk while contributing to the principles of Replacement, Reduction, and Refinement (3Rs) in preclinical drug development.

Keywords: gastro-intestinal, toxicity, organoids, NAMs

Poster 08

Do microglia modulate chemically induced neurotoxic outcomes in a hiPSC-derived 3D brain model?

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Current OECD guidelines for developmental neurotoxicity testing rely heavily on animal models, which often fail to predict human-specific outcomes. Human iPSC-derived 3D BrainSpheres (BSs), containing neurons, oligodendrocytes and astrocytes, are a promising human-relevant *in vitro* alternative. However, BSs lack microglia, the brain's resident immune cells, which regulate oligodendrocyte survival, myelin integrity and inflammatory responses. Incorporating microglia is therefore key to increasing physiological relevance and studying processes such as myelination.

BSs were generated from the SON hiPSC line, engineered to overexpress SOX10, OLIG2 and NKX6.2, essential for oligodendrocyte differentiation and maturation. Mature oligodendrocytes were detected by MBP immunostaining in 4-week-old BSs, though sparsely; myelin sheath formation is under investigation by electron microscopy. To mimic early brain development, hiPSC-derived microglia were added at day 0, at the neural progenitor stage, and maintained throughout the 8-week differentiation. During the final week, BSs and microglia-containing BSs (µBSs) were repeatedly exposed to increasing concentrations of cuprizone (CPZ) and lysophosphatidylcholine (LPC), two agents widely used to induce myelin disruption.

In BSs, CPZ and LPC reduced MBP levels by approximately 50-60% at 50 and 100 µM, as measured by ELISA, suggesting alterations of mature oligodendrocytes. In µBSs, TREM2 immunostaining confirmed that microglia remain viable throughout the 8-week culture. Ongoing work aims to assess microglial functionality and their influence on oligodendrocyte responses to demyelinating agents.

Mature oligodendrocytes can thus develop within a short time in SON hiPSC-derived BSs, providing a human-relevant platform to assess MBP changes. Microglia integration increases model complexity, offering a more predictive tool for next-generation chemical risk assessment.

Keywords: hiPSCs, BrainSpheres, microglia, demyelinating agents, MBP quantification

Poster 09 (withdrawn by author)

Poster 10

Structural and toxicological characterization of gut microbial fluorochemical metabolites

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Fluorinated compounds are prevalent in pharmaceuticals and agrochemicals, yet their metabolism by the human gut microbiota remains largely uncharacterized. This study aimed to identify gut microbial transformation of selected fluorochemicals and to compare the toxicological profile of the resulting metabolites with their parent compounds.

We investigated the biotransformation of 15 structurally diverse fluorochemicals using stool samples from healthy human volunteers in an *ex vivo* high-throughput fermentation system. Parent compound depletion over 24 h was quantified by LC-MS/MS. Metabolites were identified by combining in silico biotransformation prediction (Pickaxe, MicrobeRX) with untargeted LC-MS-based metabolomics.

Three compounds — flutamide, fluazinam and pretomanid — were consistently depleted in a microbiota-dependent manner. Nitroreduction was the primary transformation observed for all three compounds, and the microbially driven nitroreduction of flutamide to flu-6 was confirmed. The potential toxicity of both compounds was characterized by QSAR-based endpoint prediction and subsequent testing of cytotoxicity in HepG2 and Caco-2 cells, barrier integrity (TEER) in Caco-2 cells, and physiologically based kinetic (PBK) modeling. Flu-6 was less cytotoxic than flutamide in both cell lines and did not impair Caco-2 barrier integrity at non-cytotoxic concentrations. PBK-modeled systemic concentrations of flu-6 remained below thresholds of cytotoxicity.

Taken together, these findings establish an integrated *ex vivo*, *in vitro* and *in silico* approach for assessing gut microbial biotransformation of fluorochemicals and its toxicological consequences.

Keywords: gut microbiota; biotransformation; fluorochemicals; untargeted metabolomics; toxicity assessment

Poster 11

Are microglial cells directly targeted by chemicals?

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Efforts to implement human cell-based new approach methodologies (NAMs) for developmental neurotoxicity (DNT) hazard assessment have led to the establishment of an *in vitro* DNT battery covering key neurodevelopmental processes. However, this battery does not include microglia. Microglia play critical roles in neurodevelopment through their process motility, release of soluble factors and phagocytic activity. Initially regarded as passive responders to CNS cell damage, microglia are now also emerging as causative contributors to neuronal dysfunction. This study aims to evaluate whether chemicals directly affect microglial core properties, thereby impacting nervous system development and ultimately leading to neurodevelopmental disorders.

Two microglial models were characterized: human immortalized cell line (HMC3) and hiPSC-derived microglia (iMGs) generated in 10 days through SPI1 and CEBPA overexpression. Both models expressed microglial markers and responded to LPS. However, the generated iMGs progressively lost PU.1 expression, raising concerns about the stability of their microglial identity. A phagocytosis assay showed similar synaptosome uptake, but iMGs degraded cargo more efficiently than HMC3. Chemical sensitivity was assessed after 24-h exposure to paraquat, amiodarone and propylene glycol phenyl ether (PGPhE). HMC3 showed no cytotoxicity at human-relevant concentrations, except for PGPhE. iMGs exhibited cytotoxicity following exposure to all compounds at or near human exposure levels, indicating higher sensitivity to chemicals.

Transcriptomic gene set variation analysis revealed low transcriptional similarity of both models to human primary microglia compared with microglia generated using longer protocols. A longer differentiation protocol currently under testing appears to improve yield and cell quality. Further characterization and comparison of these models are needed to identify the most suitable one for detecting microglia-specific chemical toxicity.

Keywords: Microglia, Developmental neurotoxicity, *in vitro* toxicology

Poster 12

Evaluating the potential testicular toxicity of Benzophenones, Parabens, Bisphenols, Phthalates, and PFAS through 17β-HSD3 inhibition

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Endocrine-disrupting chemicals (EDCs) are proposed to contribute to the global rise of male reproductive disorders. The enzyme 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) is expressed in Leydig cells and converts androstenedione to testosterone. EDC-mediated inhibition of 17β-HSD3 can impair testosterone production during both fetal development and adulthood. Our study aimed to address the limited knowledge regarding the inhibitory effects of different EDC classes on 17β-HSD3 activity.

We investigated 18 benzophenones, 11 parabens, 21 bisphenols, 14 phthalates, and 11 per- and polyfluoroalkyl substances (PFAS) using a radiometric assay with lysates from HEK-293 cells overexpressing 17β-HSD3. Cell lysates were incubated with test compounds at 10 µM, 0.2 µM [3H]-androstenedione, and 0.5 mM NADPH under conditions yielding approximately 30% substrate conversion. Reactions were terminated by adding a methanolic solution containing 2 mM androstenedione and 2 mM testosterone. Following thin-layer chromatography (TLC), substrate conversion was quantified by scintillation counting.

Among all tested chemicals, only benzophenone-1 (BP-1) significantly inhibited 17β-HSD3 activity, with an IC50 of 0.8 ± 0.1 µM, confirming our previous findings. 2,4,4'–trihydroxybenzophenone, benzylparaben, and hexylparaben exhibited lower inhibitory potencies, with IC50 values of 7.6 ± 1.6 µM, 5.2 ± 1.4 µM, and 4.7 ± 1.8 µM, respectively. In contrast, none of the bisphenols, phthalates, or PFAS significantly inhibited 17β-HSD3 under the experimental conditions. In silico molecular docking provided a structural rationale for our findings. Based on their pKa profiles, the affinity of benzophenones for 17β-HSD3 was driven by their ionizable 4-OH group, potentially forming a salt bridge with the cofactor, while the affinity of parabens depended primarily on van der Waals interactions, with extended alkyl chains enhancing binding stability.

In conclusion, BP-1 was confirmed as a potent 17β-HSD3 inhibitor together with less potent 2,4,4'–trihydroxybenzophenone, benzylparaben, and hexylparaben. Our findings identify structural features associated with 17β-HSD3 inhibition and suggest that an ionizable head group combined with an elongated hydrophobic moiety may favor binding to 17β-HSD3.

Keywords: endocrine disrupting chemicals, 17β-HSD3, testicular toxicity, testosterone, male reproductive disorders.

Poster 13

Quantitative genomic mapping of exposure-induced oxidative DNA damage: a sequence-defined redistribution of 8-oxoG that sharpens with dose

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Oxidative stress from chemical exposure or metabolism drives DNA oxidation and genomic instability, key outcomes of toxicant action. 8-oxo-7,8-dihydroguanine (8-oxoG), the most abundant DNA oxidation product, mispairs during replication to promote mutagenic G>T transversions and is elevated in many cancers and neurodegenerative diseases. How exposure dose shapes genome-wide 8-oxoG patterns remains poorly defined.

We refined our single-nucleotide 8-oxoG mapping method, click-code-seq, adding an exogenous plasmid spike-in as an internal standard so that 8-oxoG levels are comparable across samples and doses. Applied to a potassium bromate (KBrO3) dose ladder on extracted U2OS genomic DNA, the internal standard recovers a dose-dependent rise in genomic 8-oxoG. Dose shapes not only the amount of damage but also its genomic pattern: oxidation concentrates in guanine-rich sequence and tracks guanine ionisation potential, with low-ionisation-potential guanines preferentially oxidised, and this sequence selectivity sharpens with dose. In KBrO3-exposed U2OS cells, the genomic distribution of 8-oxoG follows the same sequence and ionisation-potential preference observed on naked DNA, indicating that this intrinsic selectivity is preserved in the cellular context.

This quantitative click-code-seq framework provides a robust platform for comparative mapping of induced oxidative DNA damage across exposure conditions and dose levels, enabling systematic evaluation of exposure-dependent 8-oxoG patterns to support mechanistic studies in environmental mutagenesis and genotoxicity research.

Poster 14

Bisphenol A analogues, but not phthalates, inhibit 17β-hydroxysteroid dehydrogenase type 2, a key 17β-estradiol-inactivating enzyme

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Bisphenol A (BPA) and di(2-ethylhexyl) phthalate (DEHP) are high-production-volume industrial chemicals, used respectively in polycarbonate and epoxy resins and as PVC plasticizers. Both have raised concern because of their endocrine-disrupting properties and are now being replaced by structural and functional analogues. Although many substitutes share structural similarity with BPA or DEHP, their potential to interfere with steroid-metabolizing enzymes remains poorly characterized. Among such enzymes, 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD2) is a key regulator of estrogen action, catalyzing the oxidative inactivation of 17β-estradiol to estrone. To our knowledge, the effects of bisphenol and phthalate replacement compounds on 17β-HSD2 have not been investigated.

We therefore screened a panel of 30 bisphenols and 13 phthalates at 10 μM for 17β-HSD2 inhibition, using lysates of HEK-293 cells overexpressing the enzyme and intact HEC-1A endometrial epithelial cells expressing it endogenously. Compounds reducing residual activity below 30% were considered positive hits, and their IC50 values were determined. In lysates, 12 bisphenol analogues, but not BPA, reduced 17β-HSD2 activity, with IC50 values ranging from 69 nM to 4.8 μM. Eight of them also inhibited the enzyme in intact cells, with IC50 values ranging from 383 nM to 4.8 μM. In contrast, none of the phthalates tested, including DEHP, affected enzyme activity.

Collectively, these results identify 17β-HSD2 as a novel target of several bisphenol analogues, whereas BPA itself was inactive. Eight BPA analogues remained active in both lysates and intact cells at sub-micromolar to low-micromolar concentrations. Because the parent compound did not inhibit the enzyme, this activity may be an unrecognized liability of BPA replacements and may raise concern about regrettable substitution. As a next step, in silico molecular modeling will be used to explore the binding modes and relative binding affinities of the active compounds and identify structural determinants of 17β-HSD2 inhibition.

Keywords: Bisphenol analogues; Phthalates; 17β-hydroxysteroid dehydrogenase 2; Endocrine disruption; Regrettable substitution.

Poster 15 (selected for Short Oral Presentation)

An integrative *in silico* framework for derisking drug-induced liver injury (DILI) liabilities

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Drug-induced liver injury (DILI) is a leading cause of clinical trial failure and drug withdrawal, making early derisking essential for both drug commercialization and patient safety. Despite 0advances in new approach methodologies (NAMs), current predictive models show limited realworld 0applicability, due to industry-specific scaffolds, sparse/unharmonized preclinical data and limited clinical data. To address these challenges, we developed a machine learning (ML) driven model designed to predict DILI risk and associated clinical manifestations by systematically integrating and harmonizing physicochemical properties, pharmacokinetic (PK), *in vitro* and preclinical data.

Our approach integrates (1) external public data acquisition, (2) internal historical data identification and harmonization and (3) development of a preclinical/clinical hepatotoxicity classification. We conducted systematic literature screening and mining of public repositories (e.g. DILIrank, FAERS, ChEMBL) to identify high-quality, open access datasets. To ensure biological relevance, we focused on endpoints with a well-known mechanistic link to DILI. From 35 curated publications evaluating DILI predictivity using *in vitro* or *in silico* NAMs, we extracted key endpoints, including cytotoxicity from *in vitro* hepatic models (e.g. HepG2, hLimt, PHH), reactive metabolite formation (e.g. GSH), bile salt transport inhibition (e.g. BSEP), mitochondrial toxicity, and pharmacokinetic parameters (e.g. Cmax, Tmax, dose). We compiled data for over 1300 small molecules, from which 752 molecules contained unambiguous DILIrank labels, including clinical DILI manifestations and classification (e.g. cholestasis, hepatocellular). Additional metadata retrieved included indications, box warnings, adverse events and physicochemical properties.

Pharmaceutical companies adapt their safety strategies in response to a dynamic landscape of drug targets, modalities, regulatory requirements and novel capabilities. Building a database navigating through historical data is a challenge, requiring careful data harmonization efforts and evaluation of protocols, assay predictive variability and data interpretation rules. We systematically retraced and collected current and historical *in vitro* assays, including hepatocyte viability, bile transport inhibition and reactive metabolite formation; and produced a hepatotoxicity preclinical and clinical classification. We classified internal small molecules using DILIrank criteria and retrieved DILI clinical manifestations by analyzing raw clinical data and LLM-extracted information from historical reports. DILI Clinical classification was based on hepatic adverse event severity, Hy's Law, and CTCAE-graded hepatic laboratory abnormalities.

By integrating proprietary and external *in vitro*, PK and clinical data, we have successfully bridged critical data gaps and generated a ML-driven tool for early derisking of drug candidates, supporting the transition towards safe entry into humans while reducing *in vitro* and animal testing requirements. Furthermore, the model has the potential to classify DILI severity and elucidate underlying mechanisms based on distinct clinical manifestations.

Poster 16

Drug-induced inhibition of non-xenobiotic CYPs: effect of azole antifungals on the vitamin D synthesis

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Non-drug-metabolizing cytochrome P450 enzymes (CYP450s) are often overlooked in pre-clinical safety assessments, leading to unexpected post-marketing adverse effects. This is demonstrated for azole antifungals, which exhibit multiple off-target inhibitory effects on human CYP450s instead of solely inhibiting the fungal CYP51. To date, it is unknown whether these azoles can also inhibit CYP27B1, the only enzyme responsible for the final step of vitamin D synthesis. To address this question, a cell-free CYP27B1 enzymatic assay was conducted, followed by an ELISA to quantify the converted 1,25-dihydroxyvitamin D product. Several systemic and topical azole antifungals were screened at a concentration of 10 µM. Azoles showing inhibitory activity were further investigated to determine their IC_{50} values. All tested topical azole antifungals, namely miconazole, oxiconazole, luliconazole and econazole, significantly inhibited CYP27B1. Among the systemic azole antifungals, four out of the six tested triazoles inhibited the enzyme significantly, namely posaconazole, itraconazole, hydroxy-itraconazole and isavuconazole. None of the three tested tetrazoles inhibited the enzyme, consistent with their increasing specificity for the fungal CYP51. IC_{50} values for most observed inhibitors are in the nanomolar range, with the exception of isavuconazole and oxiconazole, which have IC_{50} values in the low micromolar range. Nevertheless, all IC_{50} values for the systemic azole antifungals are within the reported serum concentrations of treated patients. Therefore, these findings indicate that several azole antifungals can inhibit CYP27B1 at therapeutically relevant concentrations, and that CYP27B1 represents a previously unrecognized target. This could explain some of the observed adverse effects during prolonged use of azole antifungals. Future work will confirm these findings in a physiologically relevant cell line expressing CYP27B1.

Keywords: Azole antifungals, Vitamin D, CYP27B1

Poster 17

A Computational Approach to Assess the Impacts of Pesticide Exposure on Intraspecific Genetic Diversity Across Species

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Anthropogenic pressures can alter the evolutionary trajectory of organisms, affecting biodiversity at the ecosystem, species, and genetic level. While research has examined climate change and habitat loss at the species and ecosystem level, chemical impacts on intraspecific genetic diversity remain understudied. With over 350,000 chemicals on the marketplace globally, experimentally testing their impact on thousands of species is not feasible. To address this, we piloted a computational approach using publicly available data to create genetic susceptibility distributions (GSDs) for cross-species comparison of chemical impacts on intraspecific genetic diversity. This method was applied in Europe with modeled pesticide application rate maps for 103 pesticides and whole genome re-sequencing data from 6 terrestrial species as input data. An environmental association analysis (pRDA) was used to identify single nucleotide polymorphisms (SNPs) correlating with pesticide concentrations. Concentration-response curves describing the relationship between exposure concentration and the percent of affected SNPs were derived for each species-pesticide combination, then aggregated across species to describe the proportion of species with impacted genetics across a concentration gradient. This approach mirrors the ecotoxicology concept of species sensitivity distributions (SSDs), but from a genetics perspective. Our results successfully calculated EC50 values for 225 species-chemical combinations and derived GSDs for 12 pesticides. We assessed the concentration response curve predictive power and strength of chemical-species associations to provide a tiered confidence level for each chemical-species combination. Our study is the first large-scale quantitative approach to assess chemical impact on wild population genetics across species. We propose that this method can complement traditional ecosystem toxicological methods to holistically assess chemical impacts on wild populations.

Poster 18 (selected for Short Oral Presentation)

Maternal mercury exposure is associated with lower nestling mitochondrial respiration in a wild songbird: a cross-fostering experiment

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Mercury (Hg) is a global pollutant that biomagnifies in aquatic food webs and that can harm wild animals by disrupting mitochondrial function, and thereby lowering energy production for growth, reproduction and maintenance. Negative effects on offspring mitochondria and development can arise from maternal transfer of Hg before hatching or from exposure during rearing, but their relative contributions have rarely been disentangled in the wild. We addressed this with a partial cross-fostering experiment in white-throated dippers (Cinclus cinclus), a riverine songbird breeding along a natural Hg gradient in the Chartreuse Mountains in France, where parental feather Hg ranged from 0.4 to 7.2 mg/kg. Over two breeding seasons, half-broods were exchanged between 62 nest pairs two days after hatching, separating natal (genetic and pre-hatching maternal) from rearing (post-hatching dietary and parental) influences. At day 12, we blood-sampled 459 nestlings and measured mitochondrial respirations, including oxidative phosphorylation (OXPHOS), proton leak (LEAK) and coupling efficiency, using a plate-reader respirometry assay. We also measured mitochondrial content as citrate synthase activity. Only maternal Hg at the natal nest was linked to nestling mitochondria: chicks hatched from more contaminated mothers had lower OXPHOS respiration, the oxygen consumption that drives ATP production, with no change in LEAK, coupling efficiency or mitochondrial content. Our results suggest that maternally transferred Hg reduces the capacity of offspring mitochondria to produce energy, acting mainly before hatching and without reducing mitochondrial content. Lower energy production during rapid growth could constrain development and survival. Blood mitochondrial function offers a minimally invasive biomarker of sublethal contaminant effects in wildlife, and cross-fostering designs can pinpoint the developmental windows in which exposure matters most.

Keywords: mercury; mitochondrial function; maternal transfer; cross-fostering; wild birds

Poster 19

Profiles of DNA oxidation and dysregulated genome maintenance in Alzheimer's disease

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Alzheimer's disease (AD) is one of the most challenging neurodegenerative disorders to treat, due to factors like late-stage intervention and gaps in knowledge of molecular pathologies. The primary DNA oxidation product 8-oxo-guanine (8-oxoG) is elevated in brain tissue in the earliest detectable phase of AD. If unrepaired, 8-oxoG is mutagenic and associated with G>T/C>A mutations, increased in AD. While the increase in DNA damage and mutation, accompanied by altered gene expression profiles, suggests inadequate genome maintenance in AD pathology, a genomic-level understanding remains limited. To characterize oxidative DNA damage accumulation in AD, we established a method for single nucleotide resolution, strand-resolved DNA oxidation mapping, (Lefevre et al. 2026, Star Protocols; Takhaveev et al. 2025, Nature Chemical Biology) and optimized it to map oxidized bases in the brain. To understand implications of 8-oxoG enrichment in AD brains, such as a basis for somatic G>T/C>A mutations or altered gene regulation, we characterized its never-before-reported genomic distribution in post-mortem dorsolateral prefrontal cortex samples from AD patients and neurotypical controls. We could confirm 2.5-fold higher 8-oxoG in AD vs. control, with high G-content bins being more oxidized in AD than in healthy samples. Across dinucleotide contexts, we found genome-wide preferential oxidation of guanine in CpG-context in AD compared to healthy controls, and an enrichment of 8-oxoG within CpG-islands. These findings suggest a possible link between DNA methylation and oxidation, impacting genes and regulatory regions implicated in AD.

Keywords: DNA damage, oxidative damage, Alzheimer's disease,

Poster 20

From Tumor Response to Safety: Mechanistic and Predictive Toxicology within the ORION AI Microfactory

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Precision oncology aims to identify effective and safe treatments for each patient. The Innosuisse Flagship project ORION is a large consortium developing an AI-powered microfactory based on automation, patient-derived tissue, and data. It aims to generate, characterize and implement patient-specific tumor models for personalized therapeutic screening. The therapeutic focus lies on pancreatic and bladder cancer.

Within the ORION consortium, Subproject 4 (SP4) focuses on therapeutic screening, biomarker discovery and safety assessment. Because effective tumor treatment may cause damage to healthy tissues, efficacy testing is performed alongside toxicity assessment using human-relevant *in vitro* models for the intestine and liver, with plans to incorporate healthy bladder tissue in the future.

Initially, the toxicity of selected small molecules will be determined using Caco-2 cells for intestinal viability and barrier function and HepaRG cells for hepatic viability and function. Future plans include the use of advanced systems, namely HepaRG spheroids and chip-based Caco-2 formats (AlveoliX platform), to gain mechanistic insight into adverse effects associated with compounds that show therapeutic potential but unfavorable toxicity profiles.

Currently, the toxicity models are being established in a medium-throughput setting, alongside the careful selection of suitable model compounds. For compound selection, characterization and annotation, the 3RnD® platform from AsedaSciences® has been implemented. This curated platform supports compound prioritization based on the identification of potential molecular targets and existing toxicity readouts.

Findings from the toxicity screening will be compared with published toxicity data, documented clinical adverse effects, and available ORION tumor-response data to support risk assessment and define an *in vitro* therapeutic index.

Keywords: precision oncology; predictive toxicology; Caco-2; HepaRG; screening

Poster 21

Towards a structure-based framework for investigating gut microbiota metabolism of environmental contaminants

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Environmental contaminants can undergo biotransformation by the human gut microbiota, potentially altering their bioavailability, systemic exposure, and toxicological properties. However, gut microbial metabolism remains insufficiently characterized for many chemicals relevant to human exposure, limiting the integration of microbiome-mediated transformations into chemical risk assessment. Although experimental studies of individual compounds provide mechanistic insights, their findings cannot readily be extrapolated to other chemicals. A systematic, structure-based approach is therefore needed to prioritize compounds for further investigation.

As a model compound for developing a pipeline linking gut microbiota-mediated metabolism to human health risk assessment, we investigated the metabolism of the chloroacetamide herbicide metazachlor in a previously published study. We exposed human gut microbial communities to metazachlor at concentrations based on the EFSA-derived acceptable daily intake (ADI). Using untargeted LC-MS/MS analysis, we identified 5 known and 17 previously unreported metabolites of metazachlor and annotated these on the basis of putative biotransformation pathways. Selected metabolites were subsequently synthesized as authentic reference standards and used in fecal slurry fermentation experiments to investigate their formation and interconversion. These standards also enabled in vitro assessment of metabolite cytotoxicity and intestinal permeability. The resulting experimental data were integrated into a physiologically based kinetic (PBK) model to predict systemic concentrations of metazachlor and its key metabolites. To more broadly implement this strategy, we use structure-based evaluation of additional chemicals for experimental investigation. The EFSA OpenFoodTox database compiles chemical and toxicological information on substances relevant to human, animal, and environmental exposure, including pesticides, food additives, contaminants, and food contact materials. We screened candidate compounds on the basis of physicochemical properties to support systematic prioritization of diverse chemicals for addressing the impacts of microbial metabolism.

Keywords: gut microbiota, metabolism, environmental contaminants, LC-MS

Poster 22

Through the Tunnel, Toward the Heme: Why Furan Access Differs in Rat and Human CYP2E1

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Furan and alkylfurans occur in heat-processed foods. CYP2E1-mediated oxidation of the furan ring can generate reactive dicarbonyls of toxicological concern. To explore the molecular basis of differences in their metabolism between rats and humans, we compared five furans using 100 independent 400-ns molecular dynamics simulations of spontaneous entry into resting-state CYP2E1. We analysed substrate tunnels, passage from entrance halls to the catalytic cavity, distances to a projected oxyferryl oxygen, and the geometry of five conserved residues at the active-site rim. Docking into MD-relaxed Compound I structures examined possible oxidation poses.

Despite highly conserved active sites, substrate access differed between species. Human CYP2E1 showed more hall-reaching events but more stalls, whereas rat CYP2E1 transferred a greater proportion of arriving ligands into the cavity or active site. Human entries favored the cytosol-facing tunnel 2c; rat entries favored tunnel 2f, oriented toward the endoplasmic reticulum membrane. In the resting-state simulations, ring and side-chain carbons approached the projected oxyferryl position more closely in rat. Distances among conserved rim residues were generally shorter in human, consistent with more restricted access toward the heme. Docking suggested that furan and monoalkylfurans can present their rings toward the oxyferryl group, whereas the two methyl groups of 2,5-dimethylfuran can shield its ring double bonds and favor a side-chain-facing pose.

Together, tunnel selection, active-site geometry and ligand orientation provide a mechanistic framework for interpreting differences in the potential bioactivation of food-relevant furans. These geometric observations do not directly predict reaction rates.

Keywords: furans; CYP2E1; molecular dynamics; substrate access; bioactivation

Poster 23

Prednisone Exposure and Adrenal Steroid Profiles Are Associated with Relapses in PMR and GCA

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Glucocorticoids are central anti-inflammatory drugs in rheumatology. Polymyalgia rheumatica (PMR) and giant cell arteritis (GCA) are commonly treated with oral prednisone, a prodrug converted to prednisolone. Relapses remain frequent during treatment, affecting approximately 40–50% of patients. Why inflammation re-emerges despite ongoing prednisone therapy remains insufficiently understood. Potential explanations include suboptimal adherence, altered prednisone activation or clearance, or impaired glucocorticoid responsiveness.

In this exploratory study, LC-MS/MS was used to quantify prednisone metabolites and endogenous steroids in 32 PMR/GCA patients receiving 15 mg prednisone daily. Fasting serum was collected before and after standardized prednisone intake, while 24-h urinary prednisone metabolites supported assessment of treatment adherence. Relapses were monitored over the subsequent 12 months.

Patients who experienced a relapse during follow-up showed lower pre-dose prednisolone concentrations than patients who remained relapse-free (3.18 vs 6.70 ng/mL). In contrast, endogenous adrenal steroids were higher, including cortisol (165.9 vs 59.8 ng/mL), cortisone (12.8 vs 6.8 ng/mL), DHEAS (227.8 vs 70.8 ng/mL), and androstenedione (0.30 vs 0.12 ng/mL). After acute prednisone intake, serum prednisone and prednisolone increased in both groups without clear separation. The prednisolone/prednisone ratio showed no consistent differences in apparent systemic prednisone activation.

These findings suggest that relapse is associated with lower pre-dose prednisolone and higher endogenous adrenal steroid concentrations. This phenotype may reflect increased glucocorticoid clearance, altered HPA-axis feedback, or reduced glucocorticoid sensitivity.

These exploratory findings require validation in larger cohorts, including investigation of HPA-axis activity, glucocorticoid receptor expression and sensitivity, and inflammatory status.

Keywords: Glucocorticoids, Rheumatic Diseases, Biomarker, Steroids, Prednisolone

Poster 24

Integrated Assessment of Groundwater Ecosystems: Acute and Mechanism-specific Effects of Groundwater Samples from the Urban Area of Hannover, Germany

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Groundwater represents the biggest freshwater compartment in the global hydrological cycle. It provides water for half of the world's population, as drinking water and for irrigation. However, this vital resource is under great pressure, due to increased water extraction and reduced recharge, and qualitatively due to pollutant infiltration from the surface. Pollutants mainly originate from industry, wastewater discharge or agriculture, resulting in complex chemical mixtures in groundwater consisting of e.g., nitrate, pharmaceutical residues and perfluorinated substances (PFAS). Groundwater is not only a manageable resource, but also a vulnerable, inhabited ecosystem. However, effect-based ecotoxicological data, using groundwater samples or obligate groundwater species as test organisms are still very scarce. This study focuses on the acute toxicity towards a stygophilic species and the endocrine disrupting potential of groundwater samples on cellular level. Nine samples were taken from aquifers in the urban area of Hanover, Germany and extracted via solid-phase extraction (SPE). Subsequently, chemically activated luciferase expression assays (CALUX®) to detect (anti-) estrogenic or (anti-) androgenic effects were conducted. Three native samples were also tested for acute and chronic toxicity in a proof-of-concept study using the styphophilic copepod species Eucyclops serrulatus. Regarding endocrine disrupting effects detected with the CALUX® assays, eight out of nine sampling sites showed endocrine activity, mostly anti-androgenic and partly estrogenic. Endocrine activity correlates, among others, with the analgesic metabolite 2-hydroxyibuprofen, which most likely indicates the discharge of a complex mixture of EDCs via municipal wastewater. First data regarding

the acute toxicity of the three selected native groundwater samples suggest no lethal effects on nauplii or adult copepods. Further ecotoxicological relevant endpoints will be tested with the same samples and correlated to chemical and faunistic data. This study is part of the joint project "gwTriade", which aims to develop a holistic assessment concept of groundwater ecosystems based on a triad approach, combining chemical analysis, standardised ecotoxicological bioassays and faunistic diversity.

Poster 25

Decoding Toxicological Modes of Action with Metabolomics: The MetaMap®Tox Database

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Metabolomics is an emerging New Approach Methodology (NAM) that captures treatment-induced biochemical perturbations and thereby supports mechanistic toxicity assessment and the prediction of toxicological modes of action (MoAs) of chemicals.

The MetaMap®Tox database enables early characterization of potential compound-related toxicities by predicting MoAs, ranking test compounds against reference substances with similar metabolic profiles and supporting biochemical pathway analysis.

MetaMap®Tox database comprises dose-resolved plasma metabolome profiles from more than 1,100 compounds evaluated in highly standardized *in vivo* rat studies based on OECD Test Guideline 407. The database includes approximately 450 BASF-proprietary compounds and 650 additional substances, including pharmaceuticals. Integrated tools for pattern ranking, pairwise treatment correlation, statistical analysis, and comparative evaluation of significantly altered metabolites support toxicity characterization and MoA prediction.

The MetaMap®Tox database additionally contains more than 110 so-called patterns or in other words "metabolic signatures" associated with distinct toxicological MoAs, including f.ex. liver peroxisome proliferation, endocrine disruption, and kidney effects. Other patterns can f.ex. also distinguish direct thyroid inhibition versus indirect thyroid effects secondary to hepatic enzyme induction. Pattern ranking compares the metabolome profile of a test compound with these reference signatures, based on the premise that compounds that produce a specific form of toxicity also share a common set of metabolites, thereby enabling data-driven MoA identification.

The pairwise treatment correlation compares the metabolome profile of a test compound with all reference profiles in MetaMap®Tox. This approach identifies substances with similar metabolic responses and supports inference of potential toxicological effects from well-characterized comparators.

Together, these complementary analyses establish MetaMap®Tox as a robust platform for early *in vivo* toxicity characterization and mechanistically informed mode of action prediction.

Poster 26 (late submission)

Quantitative Assessment of Microglial functions for *In Vitro* DNT Batteries

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Microglia play essential roles in CNS development and homeostasis, contributing to neural circuit formation and maturation through interactions with surrounding cells and tightly regulated migratory and phagocytic responses.

Exposure to chemicals during sensitive developmental periods may disrupt microglial signaling, metabolism, and functional responses, with potential consequences for neuronal connectivity and long-term neurodevelopment. The extent to which these alterations contribute to DNT remains insufficiently defined, highlighting the relevance of microglia-specific functional assessments within emerging NAMs and the existing *in vitro* DNT testing framework.

Here, we set out to develop tests to measure phagocytosis and migration in microglia using the established cell line HMC3. Phagocytosis assays were performed using particles linked to a fluorophore, combined with immunostaining (LAMP1) to assess successful cellular uptake. While polystyrene beads and pHrodo™-labeled E. coli bioparticles led to unreliable results in HMC3, pHrodo-conjugated amine beads proved very useful: particles taken up by HMC3 could be quantified inside the cells using time-lapse imaging and flow cytometry. Results revealed increased uptake in low serum conditions. Moreover, the uptake could be blocked with cytochalasin D, indicating an active process rather than passive diffusion of the particles through the cellular membrane. This system offers great experimental flexibility to evaluate the phagocytic capacity of microglia, including control over particle size and surface functionalization, as well as the possibility to conjugate specific ligands of interest. Currently, an assay is under development to determine the ability of the HMC3 to migrate directionally.

The established method for the detection of phagocytosis is a robust quantitative readout that can contribute to the detection of chemically induced functional impairment of microglia. Further optimizing assay duration for microglial phagocytosis, chemotaxis, and cytokine release could provide a foundation for investigating the contribution of microglial dysfunction to developmental neurotoxicity (DNT).

Keywords: developmental neurotoxicity; microglia; new approach methodologies (NAMs); DNT-IVB; *in vitro* models; chemical safety assessment

Poster 27 (late submission)

From endpoints to mechanisms: A curated resource for graphene-based materials hazard assessment within the INSIGHT Project

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Graphene-based materials (GBMs) are characterized by their high surface area and honeycomb shape structure, which is responsible for their exceptional properties, such as optical transparency, mechanical strength, and high electrical and thermal conductivity. Consequently, GBMs have been heavily investigated for a wide range of areas, including electronics, energy storage, sensing technologies, environmental remediation, construction materials, tissue engineering, and drug delivery. As their applicability continues to expand toward industrial applications, there is an increasing need for the robust assessments frameworks capable of understanding their complex biological impacts.

Although a substantial amount of GBMs exposure data already exists, two major obstacles difficult their reusability in current safety assessment. First, the lack of harmonization within each individual data domain (such as physicochemical characterisation, toxicity endpoints, or omics profiles). Indeed, datasets are often highly heterogeneous and lack methodological standardisation which frequently necessitates manual curation to evaluate their usability, to enable consistent analyses, and therefore to improve their overall FAIRness (Findable, Accessible, Interoperable, and Reusable). Second, there is limited integration across domains which restricts our ability to connect material properties, toxicity endpoints, and system level biological effects.

As part of the INSIGHT Project, we present a manually curated collection for GBMs designed to address these challenges. With the aim of improving the FAIRness of existing data and enabling cross-domain integration. The core objective of this initiative is the curation, integration, and harmonisation of diverse datasets from public databases and scientific literature. This collection comprises physicochemical properties of GBMs together with a range of toxicological endpoints and transcriptomic profiles. By consolidating these heterogenous data types into a unified and more FAIR resource, this data collection supports the Safe-and-Sustainable-by-Design (SSbD) framework. Furthermore, it also facilitates hypothesis generation for hazard assessment, enabling a deeper investigation of the mechanisms underlying GBM-induced biological effects.