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Book of abstracts

ASPIS Open Symposium 2025

17 & 18 September 2025 - Athens, Greece

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The ASPIS Open Symposium 2025 will feature a total of 20 posters, highlighting a diverse range of research contributions from across the cluster and beyond. Please note that while most abstracts are included in this booklet, a few could not be shared publicly at the request of the authors.

We thank all contributors for their valuable input and look forward to an engaging and dynamic poster session.

A generalizable approach to establish neurotoxicity assays with heightened sensitivity for mitochondrial toxicants

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Conventional *in vitro* studies may lack sensitivity to mitochondrial toxicants due to the predominantly glycolytic ATP production of cultured cells in media containing high glucose concentrations. In the past, glucose (Glc) has been substituted by galactose (Gal) to make cells dependent on mitochondrial metabolism. However, not all cell culture systems and commercial media allow such a modification. Therefore we explored here the use of the glucose transporter inhibitor Glutor (GTor) as medium supplement that shifts cellular metabolism towards mitochondrial energy production. The goal was to provide a broadly-applicable strategy to improve the detection of mitotoxicants by cell-based test methods. Initially, the NeuriTox (UKN4) assay, based on human dopaminergic neurons (LUHMES) was used to evaluate shifts in metabolism and toxicity. Treatment with GTor resulted in a concentration- and time-dependent decrease in lactate production (indicator of glycolysis). Tebufenpyrad (TEBU), an inhibitor of mitochondrial respiratory chain complex-I (c-I), that is difficult to detect in glucose containing medium, was used for proof-of-concept studies. The neurotoxicity of TEBU (neurite damage) was enhanced 5000-fold (Glc BMC25= 50 µM, GTor BMC25= 10 nM) in the presence of 100 nM GTor. Similar enhancements of toxicity were observed for other c-I inhibitors. To demonstrate a general applicability, the approach was tested for various mechanistically different toxicants on LUHMES cells and also for two further (developmental) neurotoxicity assays, based on human induced pluripotent stem cell-derived peripheral neurons and neural crest cells. Thus, using GTor as cell culture supplement can be broadly applied to increase sensitivity and relevance of various cell-based assays.

AnthroDrugs-EDC: personalized EDC toxicology through population genetics

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Endocrine-disrupting chemicals (EDCs) are a major concern for human and environmental toxicology because they can cause disruptions in reproductive function, foetal development, thyroid function, and increase the risk of reproductive cancers in men and women. They are also linked to infertility, early puberty, obesity, diabetes, and various cancers (Mendes 2002, Klanova 2019) Currently marketed and new drugs may exhibit unintended endocrine disruption, which is a concern in drug design and development (Soverini 2018), since this could potentially lead to their market withdrawal or the termination of their development process. Personalized medicine is one of the elements of the P4 medicine of the future, and has already shown its ability to improve patients' lives in an individualized way (e.g., Soverini 2018, Gambardella 2020). However, a series of limitations hinder its widespread implementation in the immediate or short term, which has led to the development of different methods of population stratification (McGuire 2020, O'Hanlon Cohrt 2021, Litman 2019). One of these approaches is stratification based on the biological ancestry of patients, which has seen significant progress in recent years thanks to developments in DNA and ancient DNA analysis. Stemming from our team's previous work in personalized medicine and drug development (e.g., Font-Porterias 2021, Bhat-Ambure 2023), in this work, we propose to apply personalized toxicology for the optimization of drugs and chemicals considering their endocrine-disrupting potential by leveraging the human population genetics knowledge about genes related with endocrine disruption. We analyse drug-related genes (DRGs) which are also related to the physiological response caused by EDCs. This contributes to avoiding the current pitfalls of a drug development process specifically focused on people of European descent. In particular, we analyse a dataset of EDC-DRGs (drug toxicity-related genes) collated from the literature, describe their variability in human populations across the world, and search for potential signatures of adaptive natural selection in specific continental regions (Africa, East Asia, South Asia, Europe, and America). Thus, we analyse how population variability can affect the binding of drugs and chemicals to DRG-EDCs, allowing for the analysis of its effects on the toxicity experienced by individuals when exposed to these drugs and chemicals, and, for selected DRG-EDCs, we propose optimized drugs and chemicals, contributing to newer, optimized, safer-by-design substances for all human populations.

(Epi)genetic insights into liver injury induced by lead, cadmium, mercury, and arsenic mixture: *in silico* approach

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The liver plays a central role in the metabolism and accumulation of metals. Impaired liver function is a major contributor to the onset of conditions such as cirrhosis, hepatic steatosis, and hepatocellular carcinoma—diseases collectively responsible for nearly two million deaths annually. In clinical settings, liver injury is commonly evaluated using sensitive and specific biomarkers, including serum enzymes and proteins such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), direct and total bilirubin, indirect bilirubin, γ-glutamyl transpeptidase (GGT), and albumin. Increasing evidence from both epidemiological studies and animal models indicates a strong association between metal exposure and liver dysfunction, which may lead to either acute or chronic liver failure. Despite this, the epigenetic mechanisms underlying liver injury caused by combined metal exposures remain inadequately explored. The aim of this study was to elucidate the (epi)genetic biomarkers and mechanisms of metal mixture-induced liver injury related to lead, cadmium, mercury and arsenic co-exposure through *in silico* approach. Target genes (37) related to metal mixture-induced liver injury were obtained from Comparative Toxicogenomics Database. The enrichment analysis revealed key regulators of gene expression: miRNAs (MIENTURNET: hsa-miR-340-5p, hsa-miR-101-3p.1, hsa-miR-186-5p) and transcription factors (ChIP-X Enrichment Analysis version 3: SNAI1, IRF4, REL, DDIT3, FOSB, ZNF217, MSC, GTF2B, NR0B1, ASCL3). The main type of interaction among the investigated genes were co-expression (31.84%), interactions predicted by the server (31.77%) and physical interactions (13.90%) (GeneMANIA). Gene ontology enrichment analysis revealed (ToppGeneSuite portal): 1) molecular functions (antioxidant activity, modified amino acid binding, toxic substance binding, oxidoreductase activity, lipid binding), 2) biological processes (response to toxic substance, detoxification, cellular response to toxic substance, response to xenobiotic stimulus, circulatory system process) and 3) pathways (Nuclear receptors meta-pathway, Selenium micronutrient network, Oxidative stress response, Folate metabolism, Cellular response to chemical stress). The proposed (epi)genetic biomarkers and regulatory mechanisms associated with liver injury induced by co-exposure to lead, cadmium, mercury, and arsenic enhance the mechanistic understanding of metal mixture–induced hepatotoxicity and may serve as potential early indicators for identifying individuals at increased risk.

Rapid identification of neurotoxicity alerts for multiple compound classes by high-throughput single cell Ca2+ assays

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Neurotoxicity in adults (NT) and during development (DNT) is a major safety concern for all chemicals of the human exposome. Exposure to chemicals has been linked to the increasing prevalence of some neurodegenerative diseases and autism spectrum disorders. Currently available tests, such as the DNT *in vitro* battery (DNT-IVB) address some, but not all, functional alterations and key neurodevelopmental processes (KNDP). We utilized a single-cell Ca2+ imaging assay as an addition to the existing *in vitro* test battery (Blum *et al.* 2023) to be able to test for functional neurotoxicity. We were able to identify and quantify several marine biotoxins and the findings were confirmed by patch-clamp neurophysiology methods. We used the data to derive a point-of-departure (PoD), which can be used for next generation risk assessment (NGRA). The single-cell Ca2+ imaging assay also identified other compounds known to disturb neuronal signalling in humans. Examples were some members of the compound class of pyrethroids and several pharmaceuticals and natural products known to alter the membrane potential (like carbamazepine and lidocaine). The single-cell Ca2+ imaging assay was applicable to different neuronal cell types like central nervous system LUHMES cells, and peripheral neurons. These assays provide a valuable addition to the original DNT-IVB, as they cover some of the gaps for functional neurotoxicity and allow for rapid and economic data acquisition.

Liquid biopsy multi-omic TempO-Seq platform for safety, efficacy, and environmental exposure testing in humans using a fingerstick or animals without sacrifice

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Purpose: We developed a liquid biopsy using fingerstick blood spotted on filter paper to profile gene expression for Alzheimer's and Parkinson's diseases using the TempO-Seq® DBS assay. This method achieved high diagnostic accuracy, with 98% accuracy in Alzheimer's patients. It also offers potential for animal studies, where samples like tail nick or retro-orbital blood can replace venipuncture. The test can be self-collected in humans, offering a non-invasive diagnostic and environmental exposure screening tool. In addition to gene expression, the assay can measure DNA variants, including polygenic disease risk, and protein biomarkers, enhancing its diagnostic potential. This multi-omic approach can monitor drug treatments and assess environmental exposures linked to disease risk, making it a powerful tool for disease monitoring and public health studies.
Method: The TempO-Seq DBS assay involved spotting blood onto filter paper, drying it, and punching out areas to measure the whole transcriptome (mRNA), isoforms, miRNA, DNA variants, and protein biomarkers. Initially using Whatman Grade 3 paper, we switched to Whatman 903 Proteinsaver cards for protein assessments, as they provided equivalent gene expression data. Proteins were eluted and assayed, while other measurements were taken directly from the punched card substrate. The TempO-Seq assays used for the whole transcriptome, alternative splicing, and miRNA were based on commercial reagents. Whole blood-specific attenuators reduced gene signal from highly expressed blood genes. Alzheimer's Disease, Zepbound drug injection, and exercise were used as models to demonstrate the platform's capabilities. All samples followed IRB-approved protocols.
Results: To validate the TempO-Seq DBS assay, we tested mRNA, spliced mRNA, miRNA, and DNA variants, including 14 pathogenic variants and 17 Alzheimer's-related polygenic variants (e.g., APOE4). ELISA assays were used to measure Tau and β-amyloid proteins, and calibration curves were created. We validated the assay's protein biomarker sensitivity by spiking proteins into blood samples. The assay was then used to profile Alzheimer's patients versus controls, showing its potential for diagnosing Alzheimer's, determining immunotherapy suitability, and assessing polygenic risk. Exposure studies revealed stress responses from altitude sickness and regular hiking. Additionally, a treatment signature was identified from a subject using Zepbound injections. These results demonstrate TempO-Seq DBS's ability to assess disease, risk, exposure, and treatment from a single blood sample.

Decoding PFAS Mechanisms with High-Throughput Transcriptomics

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Per- and polyfluoroalkyl substances (PFAS) are persistent and widespread contaminants. Epidemiological studies have linked PFAS exposure to hypercholesterolemia, reduced immune responses to vaccinations and infections, and an increased risk of cancer. However, PFAS modes of action remain unclear. We analyzed gene expression profiles of human liver spheroids exposed to increasing concentrations of 24 PFAS to derive transcriptomic points of departure and benchmark concentrations (BMCs). Dose response modeling was used to identify the 250 genes with the lowest BMCs for each PFAS. Hierarchical clustering analysis was employed to reveal four functionally diverse gene sets. Each gene set was affected by a distinct group of PFAS, while individual PFAS were usually part of more than one PFAS group. The biological roles of these gene sets relate to: 1) cholesterol biogenesis and cholesterol clearance (downregulated by 7 fluorocarbon or longer PFAS), putatively through discordance of cholesterol sensing by SCAP and LXR due to membrane integration of PFAS; 2) lipolysis (upregulated by 8 carbon or shorter PFAS); 3) innate immunity (downregulated by most PFAS); and 4) adaptive immunity (downregulated by sulfonate type PFAS). The distinctions between the four PFAS groups suggest that PFAS can act through at least four independent mechanisms. The molecular characteristics of each PFAS group may provide insight into the specific molecular interactions underlying their effects on gene expression. That some PFAS congeners are included in more than one PFAS group suggests that individual PFAS can act through multiple unrelated molecular interactions. This transcriptomic analysis offers a major advancement to the understanding of the molecular mechanisms underlying the effects of PFAS exposure, and provides guidance for future work that may strengthen links between PFAS exposure and their proposed effects on human health.

In vitro/in silico integrative test battery to detect adverse outcome pathway anchored events of chemical-induced cholestatic liver toxicity

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Cholestatic liver toxicity induced by chemicals is a multisectoral concern and remains a challenge to predict. This challenge arises partly due to a lack of mechanistic understanding of this adversity. Additionally, there has been a demand to step away from animal tests towards next generation risk assessment, which utilizes *in silico* and *in vitro* approaches. To improve the mechanistic understanding of chemical-induced cholestasis, an adverse outcome pathway (AOP) network was introduced in which the links between molecular initiating events (MIEs) and key events (KEs) towards the adverse outcome are depicted. Based on this AOP network, the present study built a test battery by integrating both *in vitro* and *in silico* approaches. A total of 7 test chemicals, both cholestatic and non-cholestatic, were tested in HepaRG cells. The *in vitro* assays investigated MIEs and KEs transcriptionally and functionally. The MIE of transporter changes was investigated by analysing the gene expression levels and using fluorescent substrates. Bile canaliculi dynamics was investigated via phase contrast microscopy. The KEs of endoplasmic reticulum stress, mitochondrial impairment and oxidative stress were investigated using fluorescent probes. The *in silico* approaches comprised of quantitative-structure activity relationships (QSAR), alert-based structural activity relationships (SAR) and molecular docking of relevant protein targets. The SAR and QSAR modelling classified the test chemicals based on their potential inhibitory/substrate effects. Molecular docking simulated the binding of the test chemicals to protein structures parameterized by binding energy and interaction fraction. Results of the test battery were integrated and quantified using a weight-of-evidence approach to increase the overall relevance and application potential. Both the investigation of MIEs and KEs allowed for the identification of cholestatic chemicals. In total, 80% of the cholestatic chemicals achieved a higher number of positive results than 50% of the non-cholestatic ones, indicating a promising performance. In conclusion, this study provides an animal-free mechanism-based test battery for the prediction of the cholestatic potential of chemicals and their classification according to the level of concern.

Drosophila as a powerful genetic model for studies of Precision Toxicology

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Global industries are rapidly producing and releasing tens of thousands of chemicals, yet the effects of these molecules on environmental and human health are inadequately understood. This lack of knowledge, coupled with current mammalian testing methods that are both expensive and time-consuming, leaves a dangerous knowledge gap that must be addressed using inexpensive and high-throughput models. The fruit fly *Drosophila melanogaster* has emerged as an ideal system for studying the mechanisms by which individual chemicals alter animal behavior, physiology, metabolism, and gene expression. In this regard, Drosophila studies are uniquely situated to quickly identify the molecular targets of individual toxicants via the use of high-throughput multi-omics and an unparalleled genetic toolkit. We have screened over 200 chemical compounds for lethality and behavioral phenotypes to determine appropriate exposure conditions for metabolomic and transcriptomic analysis. Here we demonstrate the power of the multi-omics approach by analyzing adult male and female flies fed acute doses of five diverse compounds (sodium arsenite, cadmium chloride, DMSO, ethprophos, and pirinixic acid). By using a combination of transcriptomic and metabolomic methods, we identified a series of dose and time-dependent effects on metabolic pathways and gene expression networks. Our analysis revealed that chemical exposure not only activates metabolic and stress-related pathways that are known to protect flies against global toxic effects, but we also uncovered previously undescribed sex-specific responses. Notably, we observed significant changes in the expression of genes involved in oogenesis and seminal fluid protein expression, indicating that these compounds significantly affect gamete formation and function. Overall, our findings demonstrate how Drosophila can serve as a powerful model to identify the sex-specific genetic and metabolic response to toxicant exposure when applying a multi-omics approach.

Towards replacing acute toxicity in vivo testing in EU chemical safety assessments

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To this day, in the European Union (EU) the potential acute toxicity of chemicals is still mostly assessed based on results from *in vivo* testing in rodents. In July 2023, the European Commission (EC) announced the preparation of a “roadmap towards phasing out animal testing in chemical safety assessments“, expected to be published early 2026. In the context of the roadmap work, the replacement of *in vivo* testing with *in silico* predictions as the default methodology for acute oral toxicity (AOT) assessment under EU chemicals legislation has been identified as having great potential in the short term.
Here, we report the outcome of this work performed by an international expert group towards this target. First, we present results of an analysis of the legal frameworks within the scope of the EC roadmap with respect to the legal requirements/regulatory needs as well as to a possible need for updating legal or guidance texts in case *in silico* methods were to become the new standard methodology for assessing AOT. Next, possible benchmarks for acceptable predictivity of *in silico* models AOT are discussed and progress with the curation of a large AOT reference data set and the subsequent performance assessment of the CATMoS *in silico* tool (DOI: 10.1289/EHP8495) is reported as a pilot for the validation of further *in silico* tools in the future.
In addition, the presentation will provide an outlook on mid- to long-term activities envisaged under the roadmap, directed towards investigating potentially acceptable conditions for waiving AOT testing, towards complementing *in silico* methods with suitable *in vitro* test designs for out-of-domain chemicals and for developing animal-free strategies for the dermal and inhalation exposure routes as well.

Activities under the European Partnership for the Assessment of Risks from Chemicals (PARC) in support of the regulatory implementation of next-generation risk assessment (NGRA)

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Developing and implementing next-generation risk assessment (NGRA) frameworks as the default approach to human health (HH) and environmental (ENV) chemical risk assessment (CRA) requires a concerted effort by diverse actors from the chemical risk assessment (CRA) community. In this presentation we inform about ongoing work under the European Partnership for the Assessment of Risks From Chemicals (PARC; https://eu-parc.eu) to support this process. In the first part we will present the activity NGRAroute, which aims at implementing Next-Generation HH/ENV Risk Assessment as the default approach in EU chemicals legislation. NGRAroute supports the European Commission's "Roadmap towards phasing out animal testing in chemical safety assessments", *inter alia* by developing the conceptual foundations for future NGRA frameworks/workflows or by engaging in activities for the concrete replacement of *in vivo* testing, e.g., for acute toxicity testing. In the second part we demonstrate how our online knowledge management and community platform PARCopedia (https://parcopedia.eu) can help break down knowledge and language barriers between the diverse segments of the chemical risk assessment community, fostering the exchange of knowledge and enabling a joint effort for innovating chemical risk assessment and management.

Life Cycle of New Approach Methodologies (NAMs): ONTOX Contributions to Developmental Neurotoxicity NAMs

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New Approach Methodologies (NAMs) are transforming chemical safety assessment by providing human-relevant, mechanistic, and animal-free testing strategies. This poster presents a six-step framework for a structured and sustainable regulatory acceptance of NAMs, using the developmental neurotoxicity (DNT) *in vitro* battery (IVB) as a case study. The framework includes: 1. Development of robust human-relevant test systems, 2. Alignment with regulatory needs, 3. Assessment of the test method readiness, 4. Fit-for-purpose scientific validation, 5. Regulatory recognition, and 6. Public availability through contract research organizations (CRO). Within ONTOX, Work Package 9 (WP9) plays a key role in implementing this framework to advance the regulatory acceptance of DNT NAMs. WP9 researchers contribute by expanding DNT IVB assay coverage for neurodevelopmental processes (KNDP). Assays are anchored in human brain development physiological maps that illustrate the KNDPs and neural cell development. WP9 ensures regulatory alignment through engagement with OECD/EFSA/ECHA and a concerted effort with other projects. The alignment is further facilitated through the adverse outcome pathway (AOP) framework and the SCAHT AOP Hub. NAM reproducibility, robustness, and predictivity have been previously assessed. WP9 thus focused on further characterizing the biological and mechanistic relevance for the enlargement of the applicability domain and *in vitro* distribution kinetic assessment. A scientific validation to establish confidence in DNT NAMs has been conducted and data are publicly available through the ToxCast database and Biostudies repository. To facilitate broad adoption, ONTOX promotes assay standardization and FAIR data access through ToxTemps, which are included in the OECD's Initial Recommendations on Evaluation of Data from the DNT IVB. Finally, the lab-to-lab transfer of the assays is currently underway to the DNTOX CRO, ensuring that they become accessible through this spin-off company and the ONTOX Hub. This structured framework—spanning from development to commercialisation—ensures that DNT NAMs are scientifically robust and regulatory-ready. The ONTOX approach is a model for NAM development, contributing to a future of human-centric, sustainable chemical risk assessment.

Regulation of key neurodevelopmental processes by disease pathways and nuclear receptors in a human Neurosphere Assay

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Developmental neurotoxicity (DNT) testing is crucial to protect the developing human brain, yet current regulatory frameworks remain insufficient. Traditional *in vivo* models are resource-intensive, limited in human relevance, and provide little mechanistic insight. As a result, the majority of environmental chemicals—including endocrine-disrupting chemicals (EDCs)—remain untested for DNT hazards. To address this shortcoming, human-based new approach methodologies (NAMs) such as the DNT *in vitro* battery (IVB) have been developed [1]. For regulatory adoption, these NAMs must demonstrate biological and pathophysiological relevance.
The Neurosphere Assay [2], a central component of the DNT IVB, employs primary human neural progenitor cells (hNPCs) to assess key neurodevelopmental processes (KNDPs), including NPC proliferation, migration of radial glia, neurons and oligodendrocytes, neurite outgrowth, and neuronal and oligodendrocyte differentiation. In this study, we examined how nuclear hormone receptors (HRs) and neurodevelopmental signaling pathways influence these KNDPs. A test set of 31 disease pathway modulators, 14 HR agonists, and 12 antagonists was screened to evaluate concentration-dependent effects on KNDP regulation. Transcriptomic analyses of HR and pathway-related gene expression in proliferating and differentiating hNPCs were conducted and benchmarked against fetal brain data from the BrainSpan atlas.

By mapping signaling- and HR-mediated regulation of KNDPs to human neurodevelopmental disorders, we provide evidence for the human relevance and mechanistic anchoring of the assay. Notably, the assay was responsive to most tested pathways (16/18) and HRs (12/14), with exceptions including signal transducer and activator of transcription 3 (STAT3), tropomyosin receptor kinase B (TrkB), androgen receptor (AR), and estrogen receptor (ER)—highlighting boundaries of the current applicability domain. Moreover, crosstalk between HRs and developmental pathways such as Wnt and Notch was evident, emphasizing the complexity of regulatory interactions governing neurodevelopment. Oligodendrocyte differentiation emerged as the most sensitive and strongly regulated endpoint, suggesting its particular utility for detecting subtle chemical interferences. Our findings support the further development of NAMs that can identify EDCs targeting hormonal signaling pathways critical to neurodevelopment.

These results were generated in the framework of the ONTOX [3] and ENDpoints [4] projects, which aim to advance next-generation safety assessment through the integration of human-relevant mechanistic data. By delineating functional impacts of hormonal and pathway signaling on KNDPs, we reinforce the biological plausibility and mechanistic interpretability of the Neurosphere Assay. These insights enhance its regulatory credibility and contribute to advancing human-relevant, mechanism-informed DNT risk assessment.

References

[1] Crofton, K. M., & Mundy, W. R. 2021, External Scientific Report on the Interpretation of Data from the Developmental Neurotoxicity In Vitro Testing Assays for Use in Integrated Approaches for Testing and Assessment. In EFSA Supporting Publications (Vol. 18, Issue 10).

[2] Koch, Katharina *et al.* 2022, Scientific Validation of Human Neurosphere Assays for Developmental Neurotoxicity Evaluation. Frontiers in Toxicology, 4, 7.

[3] Vinken, Mathieu *et al.* 2021, Safer chemicals using less animals: kick-off of the European ONTOX project. Toxicology, 458, 152846.

[4] Lupu, Diana. *et al.* 2020, The ENDpoiNTs Project: Novel Testing Strategies for Endocrine Disruptors Linked to Developmental Neurotoxicity. International Journal of Molecular Sciences, 21(3978).

ASPIS Academy: Early-Stage Researchers in Next-Generation Toxicology

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The ASPIS Academy is an innovative platform designed to empower early-stage researchers (ESRs) and redefine the future of toxicology through targeted training, collaborative opportunities, and interdisciplinary networking. Launched in 2023 as part of the ASPIS cluster—a strategic alliance of three Horizon 2020 projects (ONTOX, RISK-HUNT3R, and PrecisionTox)—the Academy supports the EU's transition toward animal-free chemical risk assessment by equipping young scientists with the tools and skills needed to lead this transformation.

The ASPIS Academy brings together a vibrant community of over 120 ESRs, offering a comprehensive suite of initiatives. Recent highlights include the 2025 Summer School in Valencia entitled "Let's Talk About Data!", which focused on research output management, data visualization, and science communication—all crucial for driving the transition to New Approach Methodologies (NAMs).

The ASPIS Academy Mentoring Program has entered its second round, matching ESRs with senior scientists to support personal and scientific growth. A dedicated Twinning Program Manual has been developed to facilitate structured short-term research exchanges between ASPIS partners. In collaboration with VHP4Safety, a series of webinars has addressed emerging challenges and opportunities in NAMs development and application. The Academy also actively engages in public outreach through the ASPIS Academy Back to School campaign, bringing NAMs into European classrooms and sparking interest in science and animal-free research from an early age. Moreover, the Academy collaborates with other early-career networks to co-organize networking opportunities and joint sessions at major scientific conferences, providing ESRs with dedicated platforms to present their work and expand their visibility within the toxicology community.

Driven by a bottom-up, ESR-led structure and supported by senior researchers, the ASPIS Academy cultivates an inclusive and collaborative environment that values diverse expertise and promotes leadership, communication, and collaboration. It serves not only as a training platform, but as a catalyst for systemic change where young scientists are inspired to innovate, connect, and contribute to a sustainable and science-driven future in toxicology. Join us in shaping the next chapter of chemical risk assessment.

Comparative toxicogenomic profiling of imidazole compounds in HepG2 cells and zebrafish embryos

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Imidazoles represent a structurally diverse family of chemicals used as pharmaceuticals, agrochemicals, and for industrial applications. Several members of this family are under regulatory scrutiny by the European Chemicals Agency (ECHA) due to their potential endocrine-disrupting properties, reprotoxicity and carcinogenicity. In this study, we investigated whether structurally related imidazoles elicit comparable molecular responses that could support chemical grouping and risk characterization. Five imidazole derivatives were tested in two complementary models: human HepG2 cells and zebrafish (Danio rerio) embryos. Exposures were performed at equivalent effect concentrations, followed by RNA sequencing and bioinformatic analysis. Compounds fall into distinct groups based on the observed gene expression changes in both models. Across compounds and models, we observed common enrichment of transcriptional changes in genes related to steroid biosynthesis pathways, the cell cycle and vesicle formation. These transcriptional signatures are consistent with phenotypic changes, such as vesicle formation in HepG2 cells, indicating cellular stress, and altered glucocorticoid signaling activity measured by transgenic reporter zebrafish embryos. Our findings highlight conserved toxicogenomic signatures of imidazoles, providing mechanistic insights that support their evaluation as a chemical group of concern. These results may inform ECHA's regulatory efforts to assess risks associated with these structurally related compounds.

LUHMES TXG-MAPr: building a tool to predict neurotoxicity using co-expression network analysis

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Next-generation risk assessment (NGRA) requires the integration of efficient, cost-effective, and biologically relevant new approach methodologies (NAMs) into safety assessment frameworks. While toxicogenomics has potential to provide valuable mechanistic insights, current implementations primarily focus on individual gene-level changes and generic gene sets. This is despite cellular stress responses and adverse outcome pathways typically involving coordinated regulation of multiple genes in tandem, possibly in tissue- and cell-type specific manners. For liver and kidney test systems, the TXG-MAPr webtool offers an accessible and interactive platform to assess molecular perturbations induced by novel compounds and explore early events leading to toxicity. However, there is thus far no equivalent resource for interrogating neuronal test systems and neurotoxicity endpoints. Here, as part of the EFSA TXG-MAP project, we present the first neuronal TXG-MAPr, based on developing dopaminergic neurons (LUHMES d2-3). Our network is generated on the basis of exposure to 43 relevant compounds, triggering distinct cellular responses representative of specific biological perturbations. Transcriptomic responses were captured across multiple concentrations and exposure times. Using weighted gene co-expression network analysis (WGCNA), we identify functionally meaningful gene modules that capture key neurotoxic and neurodevelopmental responses. Module annotations include several well-characterized pathways including oxidative and ER stress, alongside neuronally specific ones relating to neurogenesis and neurodegeneration. To evaluate the robustness of our findings, we perform extensive preservation analyses, including to examine the influence of differentiation stages on network stability, and demonstrate the importance of carefully considering reference exposure sets in such a model. Moreover, we highlight the utility of the TXG-MAPr tool by projecting an independent external dataset onto the LUHMES-derived network. By exploring the ability of our modules to distinguish between high- and low-toxicity compounds, we showcase the applicability of such methods in early-stage compound assessment and predictive toxicology. Taken together, this work highlights the power of gene co-expression network analysis in a neuronal test system and offers a mechanistic framework for system-specific toxicogenomic evaluations in neurotoxicity research moving forward.

Generation of Kidney Organoids as an Advanced In Vitro Test System for Drug-induced Kidney Injury

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Developing *in vitro* kidney injury models remains a challenge due to the complex architecture of the organ. Here, we adapted the Takasato IPSC-derived kidney organoid protocol to generate an advanced multicellular high throughput *in vitro* test system for chemical-induced kidney injury model. We performed systematic evaluations of this test system to ensure the compatibility of the kidney organoids for toxicological purposes. Our results consistently indicated that the kidney organoids exhibited a coherent formation of nephron segments including podocytes, proximal tubule, and distal tubule. Moreover, the kidney organoids also showed clear cellular responses that reflected our understanding of *in vivo* mechanisms of nephrotoxicants e.g., cisplatin. We also employed the hiPSC endogenous CRISPR-Cas GFP-P21 reporter derived kidney organoids to capture live-temporal dynamic of the DNA damage responses in the proximal tubule upon cisplatin exposure. Altogether, we demonstrated the applicability domain of the kidney organoids for toxicological purposes with a potential as a high throughput test system to monitor chemical-induced nephrotoxicity.

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