



BOOK OF ABSTRACTS

ASPIS Open Symposium 2026

2-3 July 2026, Maastricht, The Netherlands



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The ASPIS Open Symposium (OS) is an annual meeting of the members of the ASPIS cluster and the wider scientific community, focusing on advancing the safety assessment of chemicals without the use of animal testing. The 6th annual ASPIS OS will take place in Maastricht, The Netherlands, on 2-3 July 2026, as a satellite meeting to the ESTIV Congress.



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Editorial

From scientific innovation to implementation: the ASPIS journey towards animal-free safety assessment

The ASPIS Open Symposium 2026 (2-3 July, Maastricht, The Netherlands) marks an important moment in the evolution of new approach methodologies (NAMs) and next-generation chemical safety assessment in Europe. Bringing together the three European Union's Horizon 2020-funded projects: **ONTOX**, **PrecisionTox** and **RISK-HUNT3R**, representing a combined €60 million investment across more than 70 institutions in 16 European countries and the United States, the ASPIS cluster has created a unique collaborative environment in which scientific innovation, computational approaches, mechanistic understanding, quality, education and risk assessment could develop not as isolated activities, but as interconnected components of a future safety assessment framework.

Over the past years, the discussion around NAMs has changed substantially. The question is no longer simply whether animal-free approaches can generate scientifically meaningful information. Increasingly, the challenge is how these different sources of information can be combined, interpreted and translated into decisions that are transparent, reproducible and sufficiently robust for regulatory use.

The contributions collected in this abstract book illustrate this transition particularly well.

At the experimental level, increasingly sophisticated human-relevant systems are being developed to address complex biological processes and toxicological endpoints. Several contributions focus on developmental and adult neurotoxicity, combining human neuronal and glial models, functional endpoints, transcriptomics and mechanistic interrogation. Other work demonstrates how the sensitivity and biological relevance of existing in vitro systems can be deliberately improved, for example by modifying cellular metabolism to better identify mitochondrial toxicants.

At the same time, the boundaries between experimental and computational toxicology are becoming increasingly fluid. Transcriptomic approaches, artificial intelligence, natural-language processing, quantitative systems modelling, QSARs and computational predictions are being used not merely to generate additional data, but to organise biological knowledge and support mechanistic interpretation. The ASPIS work on physiological maps, for example, demonstrates how automated text mining and ontology-supported curation can help transform the scientific literature into structured representations of human biology. Similarly, AI-supported evidence synthesis and computational modelling illustrate how information that would previously have remained fragmented can increasingly contribute to systematic and quantitative assessment.

Importantly, the abstracts also demonstrate that **NAM development alone is not sufficient**. Data must be FAIR, methods must be reproducible, models must be transparent, and the assumptions and uncertainties underlying predictions must be understood. Knowledge infrastructures developed within ASPIS therefore represent much more than repositories of information: they provide the foundations for traceability, interoperability and reproducible evidence generation required for next-generation risk assessment. Quality assurance has similarly become an integral part of the process, with systematic consideration of good in vitro method practices (GIVIMP), good data practices and appropriate principles for computational models helping to build confidence in NAM-derived evidence.

Perhaps most significantly, ASPIS has progressively moved from the development of individual methods towards the question of **how evidence should be integrated for risk assessment**. The ASPIS Risk Assessment Task Force exemplifies this evolution by bringing experts from the three projects together to compare approaches, explore uncertainty, exposure, chemical grouping and read-across, and examine how diverse evidence streams can ultimately contribute to regulatory decision-making. This collaborative dimension may prove to be one of the most important legacies of ASPIS.

A scientific transition of this magnitude also requires people. The ASPIS Academy has connected and supported more than 120 early-stage researchers through training, mentoring, laboratory exchanges, scientific communication and interdisciplinary collaboration. These researchers will carry the concepts developed within ASPIS into universities, regulatory organisations, industry and future European and international initiatives. Building this community is therefore not separate from scientific progress; it is one of the conditions for sustaining it.

Communication has been equally important. The creation of a shared ASPIS identity and coordinated engagement with scientists, regulators, policymakers, industry, non-governmental organisations and the wider public has helped transform three individual research projects into a recognisable European initiative for animal-free safety assessment.

The contributions presented at the ASPIS Open Symposium 2026 therefore represent more than individual scientific results. Together, they illustrate an emerging ecosystem in **which human-relevant experimental models, computational toxicology, mechanistic knowledge, AI, exposure and kinetic information, quality frameworks and transparent evidence integration increasingly converge**.

ASPIS has demonstrated what can be achieved when projects collaborate beyond their individual scientific objectives. The methodologies, infrastructures, networks, quality principles and - above all - the scientific community created through this collaboration provide a strong foundation for what comes next.

The ASPIS Open Symposium 2026 is therefore not simply a showcase of completed work. It is a bridge between what ASPIS has built and the next phase of the transition towards human-relevant, mechanism-based and ultimately animal-free chemical safety assessment.

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The ASPIS Academy: Empowering the Next Generation of Toxicologists

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In the context of a global shift toward animal-free toxicology, early-stage researchers (ESRs) play a central role in driving innovation through New Approach Methodologies (NAMs). The ASPIS Academy (AA) is a dedicated ESR network established in 2023 within the ASPIS cluster, which unites three Horizon 2020 projects: ONTOX (1), RISK-HUNT3R (2), and PrecisionTox (3). The AA plays a pivotal role in advancing animal-free chemical risk assessment by equipping over 120 ESRs with the skills and opportunities needed to innovate in the field of toxicology. Through a diverse portfolio of initiatives, including tailored training, mentoring, lab exchange (twinning) opportunities, and science communication, the AA fosters both scientific and transferable skill development. The Academy also embraces diversity and inclusivity, ensuring that researchers from all backgrounds can actively contribute to shaping the future of toxicology. Led by ESR representatives and supported by senior scientists, the AA fosters a dynamic environment for knowledge exchange and peer support across disciplines. It empowers its members to engage with NAMs in vitro test systems, in silico tools, omics technologies, and artificial intelligence, preparing them to lead in the transition toward human-relevant, non-animal testing strategies. This poster presents the ASPIS Academy as a model for ESR engagement and capacity-building and aims to open dialogue with other networks to explore synergies, shared challenges, and collaborative opportunities in shaping the next generation of toxicologists.

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Enhancing Mechanistic Neurotoxicity Evaluations with in Vitro LUHMES-Based Gene Co-Expression Network Analysis

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Next-generation risk assessment (NGRA) is reshaping chemical safety by integrating efficient, cost-effective, and human-relevant new approach methodologies (NAMs). Toxicogenomics offers potential to extract mechanistic depth from these assays, yet current implementations rarely venture past isolated genes and non-specific gene sets. Since adverse outcomes are frequently preceded by coordinated and tissue-specific transcriptomic perturbations, it is critical that we advance safety assessment with this context in mind. For hepatotoxicity and nephrotoxicity, the TXG-MAPr webtool provides an accessible and interactive platform to explore early events and evaluate novel compounds in liver- and kidney-based test systems. No equivalent resource currently exists for neuronal assays. Here, as part of the EFSA TXG-MAP project, we present the first neuronal TXG-MAPr, built to assess toxicity in developing human dopaminergic neurons. LUHMES (d2-3) were exposed to 42 mechanistically informative compounds across relevant concentrations and timepoints, triggering a range of distinct adaptive and adverse cellular stress responses. The resulting network, built using weighted gene co-expression network analysis (WGCNA), comprises functionally-specific gene modules capturing both pathways preserved across tissues, such as oxidative and ER stress, as well as ones specific to the neurodevelopmental setting. We validate and showcase the utility of this TXG-MAPr tool for early-stage compound assessment and predictive toxicology by projecting independent external datasets onto the LUHMES-derived network. Taken together, this work expands the TXG-MAPr framework to a neuronal test system, advancing NGRA and promoting scalable, mechanistically-grounded evaluation of developmental neurotoxicity moving forward.

Establishment of an In-Vitro Neurotoxicity Test Battery Using 2D/3D Neuronal Cell Models for the Assessment of Functional Disturbances in the Nervous System

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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 only some functional alterations in key neurodevelopmental processes (KNDP) or neuronal network function. We aimed at addressing remaining gaps. Moreover, we wanted to provide tools for a detailed screen hit follow up and toxicological characterization in human cell-based assays. Therefore, we developed several functional neurotoxicity assays to investigate some of the existing gaps in the DNT-IVB EU. These assays address different levels of complexity of functional neurotoxicity that can assess both the modulation of spontaneous network activity and the response to specific stimuli. The endpoints used are changes of [Ca²⁺]_i, receptor desensitization, changes of receptor expression, alterations of neuronal connectivity, and the toxicant-induced alterations of complex oscillatory activity in neurons. We established these assays for various neuronal cell types like human dopaminergic neurons, or hiPSC-derived peripheral neurons, and hiPSC-derived mixed cortical cultures, as well as glial cells, like hiPSC-derived astrocytes. The functional neurotoxicity battery consists not only of 2D cell culture models but also incorporates 3D neuronal spheroids to increase the biological coverage. With these assays, it is possible to cover a wide range of different chemicals (e.g. neonicotinoids, pyrethroids, strobilurins and methylpyrazole pesticides). The battery identifies chemicals targeting several receptor classes like NMDA, glutamate, nAChR, purinergic receptors, as well as various ion channels like voltage-gated sodium channels (VGSCs). Overall, the first results provide a valuable addition to the original DNT-IVB to cover some of the existing gaps for functional neurotoxicity.

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 Gtor (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 mitotoxins by cell-based test methods. Initially, the NeuroTox (United KingdomN4) 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 μ M, 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.



Knowledge Infrastructure Supporting Next Generation Risk Assessment

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The transition toward Next Generation Risk Assessment (NGRA) and the growing use of New Approach Methodologies (NAMs) require robust knowledge infrastructures (KI) capable of integrating diverse experimental and computational data into transparent, reproducible, and regulatory-relevant workflows. However, toxicological data remain fragmented across heterogeneous sources, limiting interoperability, reuse, and predictive modelling. Here, we describe the design and deployment of a FAIR-compliant knowledge infrastructure developed within the RISK-HUNT3R project to support integrated data management, computational modelling, and mechanistically informed risk assessment. The infrastructure integrates harmonised data templates, curated compound data, standardised method descriptions, ontology-driven metadata annotation, knowledge graphs, and cloud-based workflow services. These components enable structured data capture, validation, and integration of experimental and computational datasets, facilitating reproducible analyses and transparent evidence generation. Ontology-linked templates, knowledge graphs, and computational workflows collectively enhance semantic interoperability, support linkage to mechanistic frameworks such as Adverse Outcome Pathways (AOPs), and enable efficient model development, validation, and application within NGRA strategies. The platform has been successfully deployed across distributed project partners and supports collaborative data curation, analysis, and regulatory-oriented reporting. This work demonstrates how FAIR-compliant KI can strengthen computational toxicology by improving data quality, interoperability, traceability, and reproducibility, while providing a scalable foundation for integrating NAMs, artificial intelligence, and mechanistic modelling into next-generation safety assessment. The approach advances human-relevant, animal-free risk assessment and supports scientific and regulatory adoption of predictive toxicology methods.

R-ODAF-Shiny: An Interactive and Reproducible Transcriptomics Analysis Framework for Regulatory Application

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Transcriptomics is increasingly used in mechanistic toxicology and NGRA, but RNA-seq results can vary depending on preprocessing, filtering, and statistical choices, affecting DEG lists, inferred mechanisms, and cross-study comparability. To improve reproducibility and accessibility, we developed R-ODAF-Shiny, a graphical interface implementing the fixed differential-expression analysis layer of R-ODAF. Starting from preprocessed count matrices and metadata, the app applies locked R-ODAF criteria for coverage, expression filtering, false discovery rate, and PCA-based outlier detection, while generating DEG tables, PCA plots, heatmaps, volcano plots, gene-level summaries, run summaries, and session information. Using an RNA-seq dataset from an ONTOX study based on platinum-compound exposure in a human proximal tubule in vitro model, R-ODAF-Shiny reproduced the reference R-ODAF workflow with identical DEG membership, retained samples, log₂ fold changes, and adjusted p values. R-ODAF-supported workflows have already contributed to NAM-related studies, including compound classification, liver steatosis, kidney toxicity, intestinal toxicity, PFAS transcriptomics, and transcriptomic point-of-departure analyses. By providing the same fixed analysis logic through an accessible interface, R-ODAF-Shiny can support future NAM studies that require harmonized, reproducible, and transparent transcriptomic analysis across different model systems. Overall, R-ODAF-Shiny lowers the technical barrier to regulatory-aligned transcriptomics analysis for NAM- and NGRA-oriented toxicology.



Estimation of Intra-Specific Ranges of Genetic Susceptibility to Toxicity Using Genetically Diverse Populations of Human Lymphoblastoid Cell Lines

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Aim of the study is to provide a greater understanding of the genetic sources of variation that underlie individual susceptibility to toxicity. Consequently, we have carried out high throughput screening of thirteen chemicals (Cadmium chloride, Sodium (meta)arsenite, Acrylamide, 4-Methyl imidazole, Dexamethasone, Cyclosporin A, Tamoxifen, Genistein, Rotenone, Tetraethylthiuram disulfide, Valproic acid, 5-fluorouracil and DMSO) in 261 lymphoblastoid male and female cell lines from 18 populations to discover genetic variability and sex-dependent changes in toxic responses in humans. Clear response differences to all toxicants were found and ANOVA analysis of the cell line dose-response toxicity data from the populations revealed significant intra-population sex differences and population specific responses to the toxicants studied. Further aim is to evaluate the degree of certainty in causal links between molecular key events and chemical risks to human health. Thus, the role of single nucleotide polymorphisms in the toxic responses will be studied using a genome wide association analysis and expression quantitative trait locus mapping is carried out to reveal phenotypic variation in the cell lines. It is commonly considered that pathways predictive of human toxicity are restricted to closely related species (i.e. mammals). By measuring variability in gene expression among non-sentient organisms (NSOs), we will demonstrate that toxicity responses are shared in distantly related organisms. Omics experiments are planned to confirm that the genetic regions controlling variation in molecular key events observed in humans also control variation in the fruit fly. With these findings we hope to promote the use of NSOs to predict human toxicity in regulatory risk assessment.

The study is conducted within the H2020 funded PrecisionTox project (Grant agreement ID: 965406).

Understanding Toxicity by Descent in PrecisionTox Transcriptomes

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The evolutionary conservation of molecular networks that regulate how cells respond to chemical exposures across species forms the basis of the evolutionary toxicology principle of toxicity by descent. This principle provides the rationale for predicting human gene expression by examining how genes respond to chemical perturbations in model biological systems, allowing non-human data to be leveraged to estimate human responses. Harnessing these ideas to predict human drug and toxicant effects while reducing the need for animal testing represents the overarching goal of the PrecisionTox (PTox) project—a toxicological research initiative funded by the European Commission. Using PTox data generated in two phases, pilot and production, that comprise whole-body RNA-Seq at multiple timepoints and two treatment doses across five model organisms plus the HepG2 cell line, we carry out differential gene expression analysis (DEA) followed by mapping to human orthologs to identify an initial set of responder genes. To explore the data, we employ visualisation and dimensionality reduction techniques to compare sample distributions before and after treatment, and perform variance partitioning to quantify the relative contributions of species and compounds to the observed gene expression variation. In addition, we learn vector representations (embeddings) of compounds from their transcriptional profiles across these phylogenetically distant model organisms. These embeddings are used to cluster compounds into putative mechanisms of action (MoA) groups, capturing shared patterns of cellular toxicity and enabling exploratory analyses such as visualization, prediction tasks, and comparison with known chemical classes. Together, our analyses lay the foundations for a cross-species transcriptome comparison framework and reveal that both shared and species-specific transcriptional responses are prevalent under chemical perturbation.

Comparative Single Cell Transcriptomics of Daphnia and Biomedical Models to Uncover Evolutionarily Conserved Tissue-Specific Responses to Environmental Toxicants

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Traditional human chemical safety testing relies primarily on rodent models due to our shared mammalian biology. However, the importance of mammalian systems is being challenged by ethical and scientific considerations. Comparative evolutionary biology demonstrates that disease-associated genes are deeply conserved across metazoans. Comparative transcriptomics can be employed to improve our understanding of evolutionarily shared responses to toxicants and broadens the utility of alternative models in human health. Toxic compounds such as industrial chemicals, pesticides and domestic chemicals, among others, can perturb metabolic processes in humans and mammals. These compounds have been strongly associated with fatty liver disease, steatohepatitis, fibrosis and in rodents, hepatocellular carcinoma. The molecular networks for liver fatty acid metabolism are regulated through the transcription factor, peroxisome proliferator-activated receptor α (PPAR α). Using comparative bulk transcriptomics, and pathway enrichment, we find that the microcrustacean Daphnia and mouse hepatocytes signal the same human pathways, regulated by PPAR α , when exposed to a potent PPAR α agonist, pirinixic acid. These networks may be evolutionarily conserved across vertebrates and invertebrates, and possibly predictive of adverse health in humans. Subsequently, we exposed Daphnia and Zebrafish to the PPAR α agonist and employed single cell RNA sequencing. We aim to uncover homologous tissue types across these models and Humans. These homologous tissue types serve as a basis for uncovering the shared tissue specific responses to pirinixic acid across vertebrates and invertebrates. This research shows promise for improving the utilisation of alternative, non-animal models for chemical safety testing of liver toxicity.



Accelerating Physiological Map Construction Through Natural Language Processing-Assisted Text Mining and Curation

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Within ONTOX, we successfully developed five organ-specific Physiological Maps (PMs) focusing on critical biological processes: bile secretion & lipid metabolism (liver), nephron physiology (kidney), neural tube closure, and cognitive function development (brain). Their creation was based on extensive manual curation of existing resources and annotation, which are time-consuming and demand a huge effort from the curation team. To address these sustainability challenges, we developed a workflow that streamlines the PM development and updating process. Our approach leverages INDRA (Integrated Network and Dynamical Reasoning Assembler) for automated text mining of causal relationships from systematic queries in PubMed. It begins with a list of PubMed IDs as input to a Python script that accesses and downloads freely available full texts as XML. INDRA uses natural language processing engines to mine these documents for molecular relationships, assigning belief weights based on the frequency of statements across papers, thereby increasing confidence in the extracted relationships. A key strength is the harmonisation and grounding of molecular entities, phenotypes, chemicals, and diseases with ontology identifiers, ensuring unambiguity in the network. Following automated extraction, manual revision occurs through a curation log to eliminate incorrectly identified causal relationships. The result is a fully annotated network using SBGN Activity Flow, which is compliant with CellDesigner SBML and GPML (WikiPathways) formats, significantly accelerating the building of PMs while maintaining high standards of accuracy and biological relevance. In the future, we plan to integrate our workflow with OmniPath, another versatile tool from the systems biology community. This integration will ensure comprehensive coverage of genes of interest while preserving the human relevance of the maps, thereby expanding their utility across diverse user groups and research applications.

A Modular Modeling Framework for Proximal Tubule injury: Towards AOP Integration

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Objective We demonstrate how the modular implementation of a published cardiovascular-renal QSP model [1] in MoBi [2] enables seamless coupling with pharmacokinetic (PK) models, facilitating detailed mechanistic investigation of renal-cardiovascular feedback, antihypertensive therapy, and drug-induced renal toxicity. We further conceptualise how quantitative AOPs (qAOPs) could be mapped onto mechanistic models to support in vitro to in vivo extrapolation. **Methods** An existing QSP renal-cardiovascular model [1] was implemented in MoBi [2], exploiting MoBi's modular architecture to enable flexible model extension and straightforward coupling to PK models. The framework was expanded with a mechanistic module for drug-induced proximal tubule injury, capturing its effects on renal function and DIKI biomarkers [3]. Simulations and visualizations were performed using the open-source ospsuite-R [2] and esqlabsR packages. The model explicitly accounts for renal hemodynamics, sodium handling, and systemic cardiovascular regulation, enabling exploration of pharmacodynamic responses across multiple biomarkers. **Results** A PK model for gentamicin was developed and integrated with the renal-cardiovascular model, incorporating proximal tubule injury. The assembled framework was applied to study gentamicin-induced PT injury, predicting changes in renal biomarkers and cardiovascular variables. Local sensitivity analysis identified parameters driving the most pronounced changes in DIKI biomarkers. This exploratory analysis is complemented by a conceptual mapping of key events (KEs) from AOP257 (<https://aopwiki.org/aops/257>) — describing KEs of aminoglycosides such as gentamicin [4] — onto the mechanistic model, bridging the fields of QSP/T and statistical learning. **Conclusion** We present a flexible, modular renal-cardiovascular modeling platform for investigating cardiovascular and renal disease, evaluating antihypertensive therapies, and assessing drug-induced kidney injury. We further outline a conceptual approach for integrating qAOPs with mechanistic modelling. By combining physiology, pharmacokinetics, and mechanistic injury pathways, the platform offers a predictive framework for simulating treatment effects across multiple biomarkers.

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The Way Out: Implementation of an ECCS-Based Tool for Predicting Xenobiotic Major Elimination Pathways

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The Extended Clearance Classification System (ECCS) predicts the predominant elimination pathway of a compound based on three properties: ionization state, molecular weight (MW), and passive membrane permeability. Although ECCS offers mechanistic insights, its practical application has been limited by the requirement for experimentally determined parameters. Here we present ECCS-DT, the first openly accessible web-based implementation of the ECCS decision tree, integrated into the ChemoPredictionSuite platform (<https://chemopredictionsuite.com/ECCS-DT>). The tool assigns organic molecules to one of the six ECCS classes by automatically calculating the necessary parameters when experimental values are not provided: MW using RDKit, ionization state at pH 7.4 using Dimorphite-DL, and MDCKII-LE passive permeability through a dedicated quantitative structure–activity relationship (QSAR) model. External validation of the QSAR model demonstrated accuracy, sensitivity, and specificity exceeding 65%. The complete workflow was validated against two independent external datasets. The complete workflow was validated against two independent external datasets: the dataset published by Varma in 2015, in which the ECCS framework was initially described, composed of pharmaceuticals; and the dataset collected within the European project ONTOX, composed of pharmaceuticals and other chemicals. At the six-class level, exact match rates were 60%59.1% and 55.7%54.8%. These rates increased to 72.3%72.6% and 60.3%59.5% when classes were grouped by predominant elimination mechanisms, and further to 79.5%79.2% and 69.2%69.6% when the composite nature of class 3B (hepatic uptake / renal clearance) was considered a valid assignment. Error analysis indicated that most misclassifications originate from the permeability prediction rather than from the ionization state assignment. ECCS-DT converts a classification framework into a fully predictive, freely available workflow for early mechanistic screening in pharmacokinetics and toxicology.

AI-Supported Analysis of DILI Phenotypes Reveals Drug-Specific Clinical Patterns While Steatosis Remains Asymptomatic

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Idiosyncratic drug-induced liver injury (DILI) is highly heterogeneous, and the contribution of hepatic steatosis to its clinical expression and outcome remains unclear. Artificial intelligence (AI)-assisted evidence synthesis offers new opportunities to efficiently identify, curate and analyse large collections of published DILI cases. We systematically collected amiodarone (AMI)-, methotrexate (MTX)- and valproate (VPA)-associated DILI case reports from PubMed using Sysrev, an AI-supported systematic review platform. Sysrev was used to assist article screening, eligibility assessment and structured data extraction, enabling the creation of a curated case-level database. This AI-enabled evidence synthesis facilitated the analysis of 187 patients and approximately 50 clinical, biochemical and histopathological variables per case (AMI n=68, MTX n=50, VPA n=69 paediatric cases). The overall prevalence of steatosis was 41% for AMI, 52% for MTX and 81% for VPA. Steatotic (n=110) and non-steatotic (n=77) DILI patients showed remarkably similar demographic, clinical, histological and routine laboratory profiles, and unsupervised multivariate analysis did not identify a distinct “steatotic DILI” phenotype. However, steatosis was associated with higher fatality (60% vs. 36%) and lower recovery rates (25% vs. 50%), an effect mainly driven by VPA and MTX but not by AMI. Comparative analyses revealed clear drug-specific DILI phenotypes: AMI in older adults with a steatohepatitic, Mallory–Denk-rich pattern; MTX with very long latency, macrovesicular steatosis, advanced fibrosis and chronicity; and paediatric VPA cases with short latency, severe necroinflammatory and cholestatic injury, coagulopathy, lactic acidosis and the highest mortality. Within established DILI, steatosis is largely clinically “silent” but may worsen outcomes in a drug- and context-dependent manner, while the causative agent remains the main determinant of the DILI phenotype.

ASPIS Communication and Dissemination: Building a Unified European Voice for Animal-Free Safety Assessment

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The ASPIS cluster, comprising the Horizon 2020 projects ONTOX, PrecisionTox, and RISK-HUNT3R, represents a €60 million European investment dedicated to advancing animal-free chemical safety assessment through New Approach Methodologies (NAMs) and Next-Generation Risk Assessment (NGRA). Beyond scientific innovation, ASPIS established a coordinated communication and dissemination framework designed to maximise visibility, stakeholder engagement, and regulatory impact across the three consortia. The ASPIS Communication and Dissemination Working Group (C&DWG) was created to harmonise outreach activities and develop a common identity capable of amplifying key messages beyond individual project boundaries. Through a shared visual identity, joint website, coordinated social media activities, policy briefs, newsletters, press releases, factsheets, public events, and stakeholder-focused communications, ASPIS successfully built a recognisable cluster brand and strengthened engagement with regulators, policymakers, industry, academia, non-governmental organisations, and the wider public. A major achievement of the cluster was the establishment of the ASPIS Academy, which provided training opportunities, networking activities, and interdisciplinary capacity building for early-career researchers. In parallel, annual ASPIS Open Symposia evolved into a key European forum where scientists, regulators, industry representatives, and policy stakeholders could discuss progress towards regulatory acceptance of NAM-based approaches. This poster highlights the communication and dissemination strategy developed across ASPIS, summarises its achievements, and reflects on lessons learned from five years of coordinated outreach. The ASPIS experience demonstrates how strategic communication can accelerate knowledge transfer, strengthen stakeholder trust, and support the translation of scientific innovation into regulatory and societal impact.

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ASPIS Risk Assessment Task Force: Advancing Next-Generation Risk Assessment Through the Experts' Collaboration

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The ASPIS cluster, comprising the Horizon 2020 projects ONTOX, PrecisionTox, and RISK-HUNT3R, was established to accelerate the transition towards animal-free chemical safety assessment through the development and implementation of New Approach Methodologies (NAMs). While each project pursued distinct scientific strategies, the ASPIS Risk Assessment Working Group (RAWG), later renamed to ASPIS Risk Assessment Task Force (RATF), was created to ensure scientific alignment, foster collaboration, and promote convergence towards practical Next-Generation Risk Assessment (NGRA) solutions. The ASPIS RATF serves as a cross-project platform for exchanging knowledge, comparing and sharing methodologies, identifying strengths and limitations of individual approaches, as well as developing joint activities and case studies. Particular emphasis is placed on hazard identification and characterisation, chemical grouping and read-across, exposure considerations, uncertainty evaluation, and the integration of diverse evidence streams into regulatory decision-making frameworks. The working group critically evaluates ASPIS developments against previous European initiatives and emerging international activities, while maintaining strong interactions with regulatory stakeholders and the European Partnership for the Assessment of Risks from Chemicals (PARC). One of the key contributions of the ASPIS RATF activities is the evaluation of the Alternative Safety Profiling Algorithm (ASPA), a structured framework designed to support the practical implementation of NAM-based risk assessment. In addition, the RATF evaluates how AI tools and platforms developed across the ASPIS projects can complement frameworks such as ASPA. A third focus area is the integration of non-sentient data to enhance the kinetic and metabolic profiling of compounds assessed solely using human-relevant in vitro and in silico NAMs. Through discussions focussed on constructive feedback, comparative evaluations, and coordinated case studies, the TF helps establish common principles for evidence integration and interpretation across the ASPIS projects. This presentation provides an overview of the objectives, activities, and achievements of the ASPIS Risk Assessment Task Force and reflects on lessons learned from five years of collaboration. The experience demonstrates how coordinated scientific efforts can support the transition from methodological innovation to regulatory application, contributing to the broader European vision of sustainable, human-relevant, and animal-free chemical safety assessment.

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WP6 - Ensuring Research Integrity in ONTOX Through Systematic Audits of In Vitro and In Silico Workflows.

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The ONTOX project, funded under the European Union's Horizon 2020 programme, aims to advance innovative, animal-free approaches for chemical risk assessment. In its original proposal, ONTOX committed to ensuring that all research activities are conducted in compliance with Good Data Practices (GDP) and Good In Vitro Method Practices (GIVIMP), thereby guaranteeing scientific robustness, transparency, and regulatory relevance of the generated outputs. To deliver on this commitment, the consortium has established a dedicated audit framework to systematically evaluate the implementation of these practices across participating research teams. Two complementary audits will be performed. The first will focus on the in vitro experimental work, assessing adherence to OECD GIVIMP principles, with particular attention to quality assurance, traceability, documentation, and method performance standards in the application of advanced 3D cell and tissue models. This audit will support the reproducibility and reliability of in vitro findings and ensure their suitability for regulatory acceptance. In parallel, a second audit will target the in silico components of ONTOX, which include computational modeling, QSARs, and other predictive tools developed in accordance with OECD principles and guidelines for model validation and use. This process will evaluate data integrity, transparency of algorithms, reproducibility of predictions, and compliance with international standards, thereby strengthening confidence in the outputs of the modeling teams. Both audits will be conducted by a dedicated ONTOX quality teams, working closely with the respective research groups to identify strengths, gaps, and opportunities for continuous improvement. By applying harmonised audit strategies to both in vitro and in silico work packages, ONTOX sets a precedent for integrated quality management across diverse scientific approaches. Ultimately, this initiative ensures that ONTOX's research outputs are generated under a framework of accountability and best practice, reinforcing their scientific credibility and regulatory applicability. The dual audit system represents an innovative step towards building trust in New Approach Methodologies (NAMs) and highlights ONTOX's leadership in promoting a culture of quality in next-generation risk assessment.

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Characterization of Opioid Receptor Function in a Human iPSC-Based Co-Culture of Astroglia and Neuronal Subpopulations Undergoing Differentiation: Case Study on Methadone and Buprenorphine

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Within the EU funded project ONTOX, researchers contribute by expanding the developmental neurotoxicity (DNT) in vitro battery (IVB) assay coverage for human key neurodevelopmental processes (KNDP) including cell maturation, synaptogenesis and astrocyte development, closing critical biological uncertainties in the current battery. Furthermore, there is an expectation that the applicability domains of the in vitro models are well characterized. Heroin abuse among pregnant mothers is a societal problem, and use of opioids like long-acting methadone or buprenorphine is recommended to treat heroin addiction (Opioid Maintenance Therapy, OMT). However, despite benefits of OMT for the mother (ie reduction of drug use, overdoses, deaths and criminality, improvement of quality-of-life etc), these opioids cross the placenta and are distributed in the fetus, and the brain develops in a potentially harmful environment. We hypothesized that exposure to methadone and buprenorphine leads to disturbed KNDP vital for healthy brain development. The current study was conducted to characterize the presence and function of the u-opioid receptor in a 2D human neural progenitor cell (NPC) model, derived from IMR90 human induced pluripotent stem cells (hiPSCs), undergoing differentiation for up to 28 days using RNA sequencing and high content imaging (HCI) (protein marker quantification). Secondly, to assess whether the NPCs are fit-for-purpose to test effects of u-opioid receptor agonists we tested repeated exposures to methadone and buprenorphine. Characterization by RNAseq enrichment analysis from four different databases revealed that the more enriched terms were related to neurodevelopment and function. Transcriptomics data showed that gene markers for astrocytes and neuronal subpopulations (glutamatergic, GABAergic, serotonergic, cholinergic, noradrenergic, glycinergic and dopaminergic) are present in the cultures at all differentiation timepoints. Presence of astrocytes in the co-culture were shown. HCI confirmed presence of u-opioid receptors in the NPCs at all differentiation timepoints and a concentration-dependent decrease in cell viability after exposure to methadone and buprenorphine was shown. Preliminary data show more dysregulated genes related to neurodevelopmental processes after exposure to buprenorphine than methadone, and both opioids showed disturbed synaptogenesis shown by HCI. This study enhances our understanding of the applicability domain for this 2D NPC model including fit-for-purpose to test u-opioid receptor agonists and thereby contributing to its regulatory readiness. Exposure to the opioids methadone and buprenorphine resulted in neurodevelopmental toxicity at low concentrations at all differentiation stages tested.

Human iPSC-Derived Glial Cells for Functional inflammatory Assays and Toxicity Testing

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Microglia and astrocytes are always activated in brain toxicities and neurodegenerative pathologies. However, data on compounds affecting glial inflammatory responses and cell-based platforms to test for this are scarce. Here, we developed an NF κ B translocation assay to capture glial responses to inflammatory stimulation and their modulation through drugs or toxicants. We utilized human iPSC-derived microglia and astrocytes exhibiting glial-specific characteristics (Wolfbeisz et al., 2025; Spreng et al., 2022). NF κ B translocation was selected as assay readout since many inflammatory pathways converge on this key event. Various TLR ligands and cytokines were applied to induce nuclear NF κ B translocation, and optimal concentrations and time points were experimentally defined. Positive controls were established, and reference compounds including IKK and proteasome inhibitors were evaluated. Additional viability assays ensured non-toxic working concentrations. Our findings highlight the utility of the NF κ B translocation assay in human glial cultures as a platform for screening compounds that modulate glial inflammatory signaling.



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